

***Cauliflower mosaic virus*, transmission and tubulin**

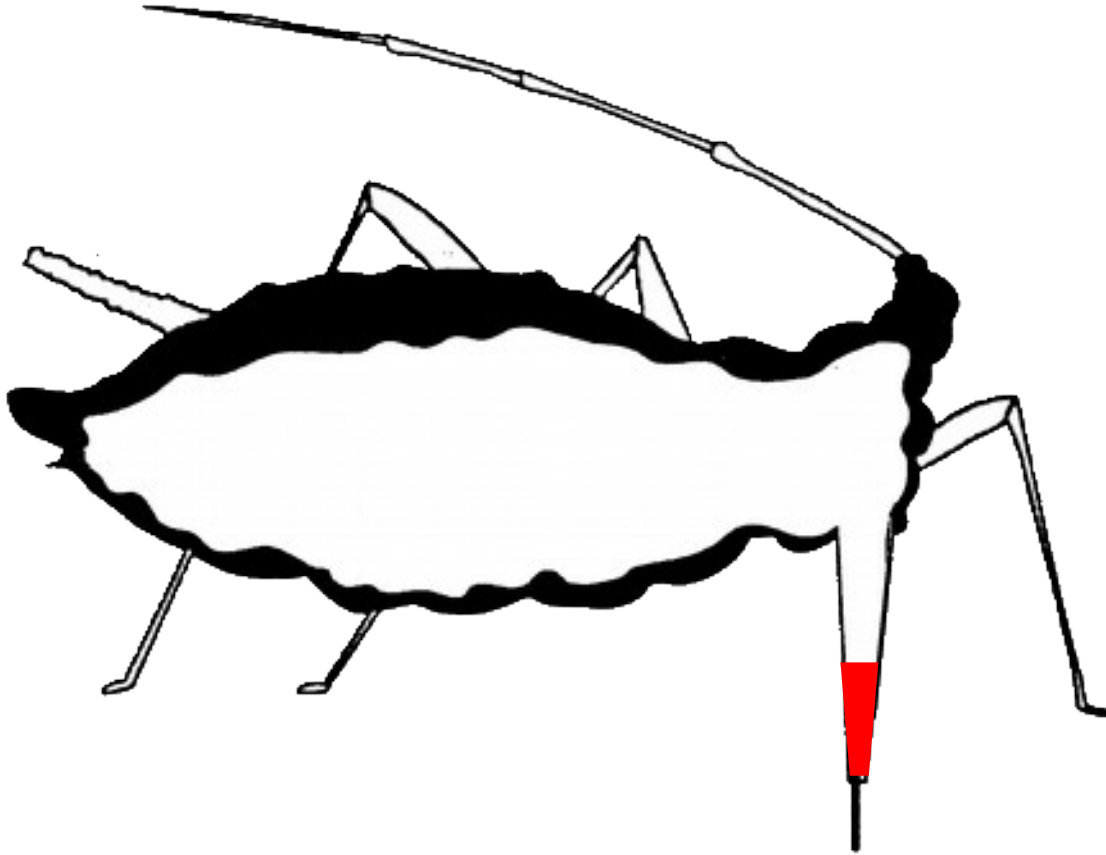
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UMR Biology of Plant Pathogen Interactions, Campus International de Baillarguet, Montpellier, France
(a) CRIC/IURC, Montpellier, France





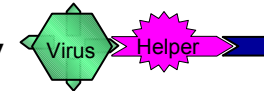
Non-circulant transmission



Capsid strategy

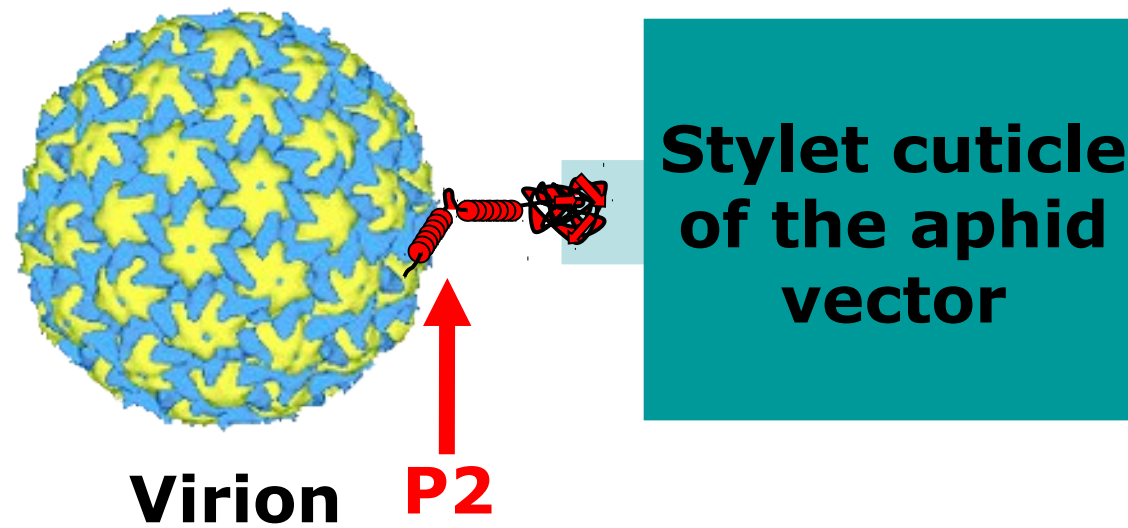


Helper strategy

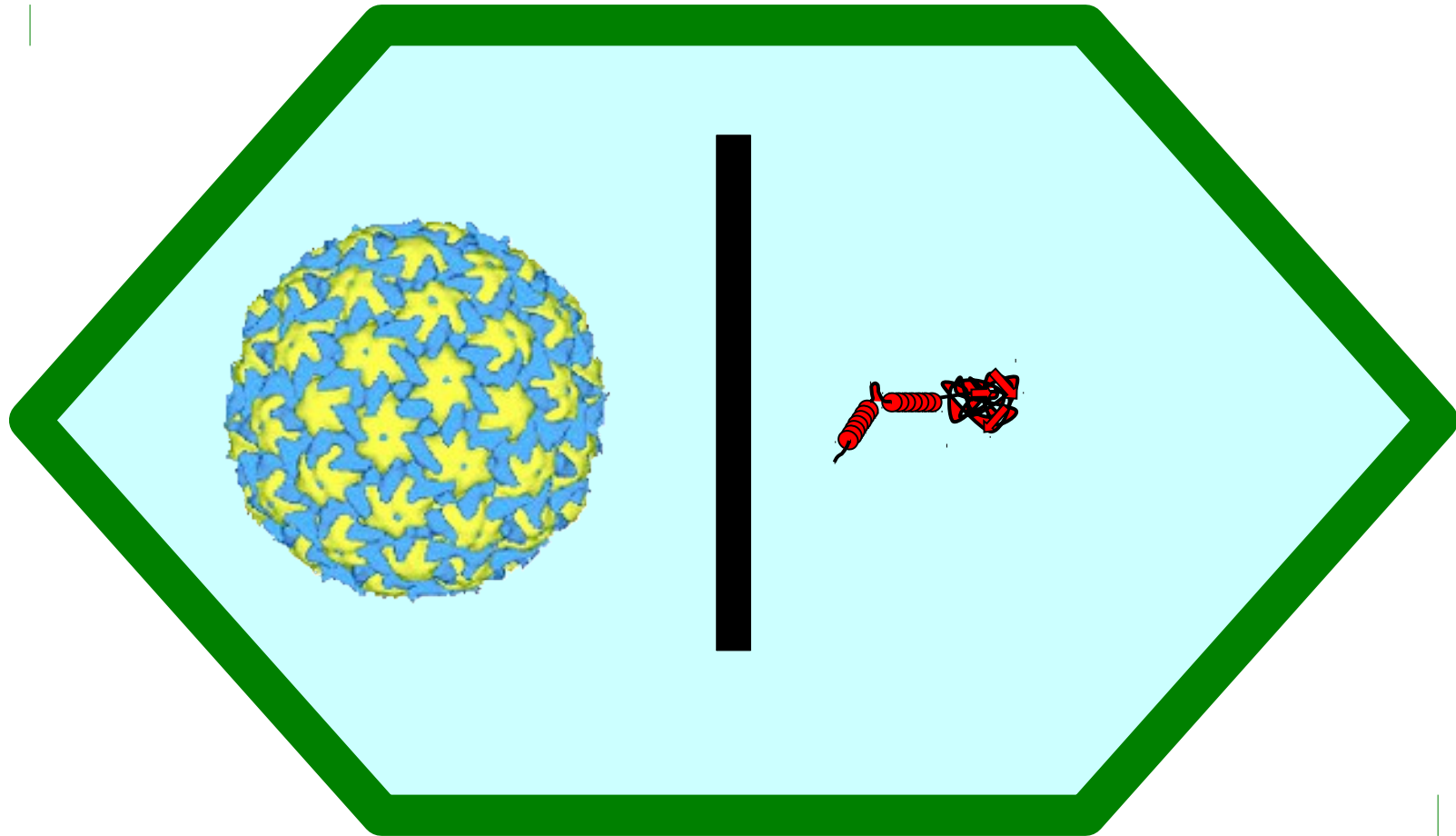


Stylet
cuticle

The CaMV transmission complex

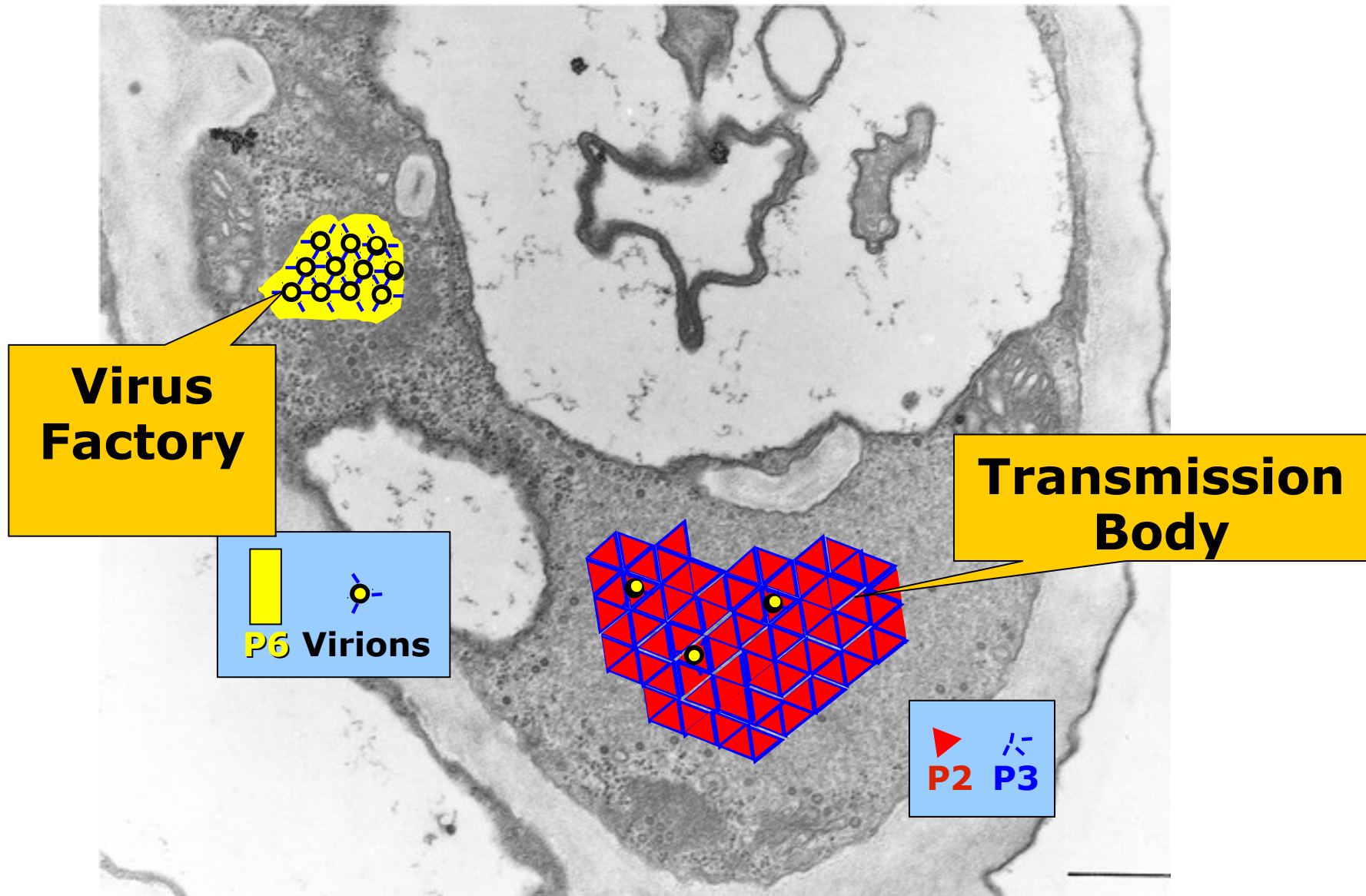


The CaMV transmission complex

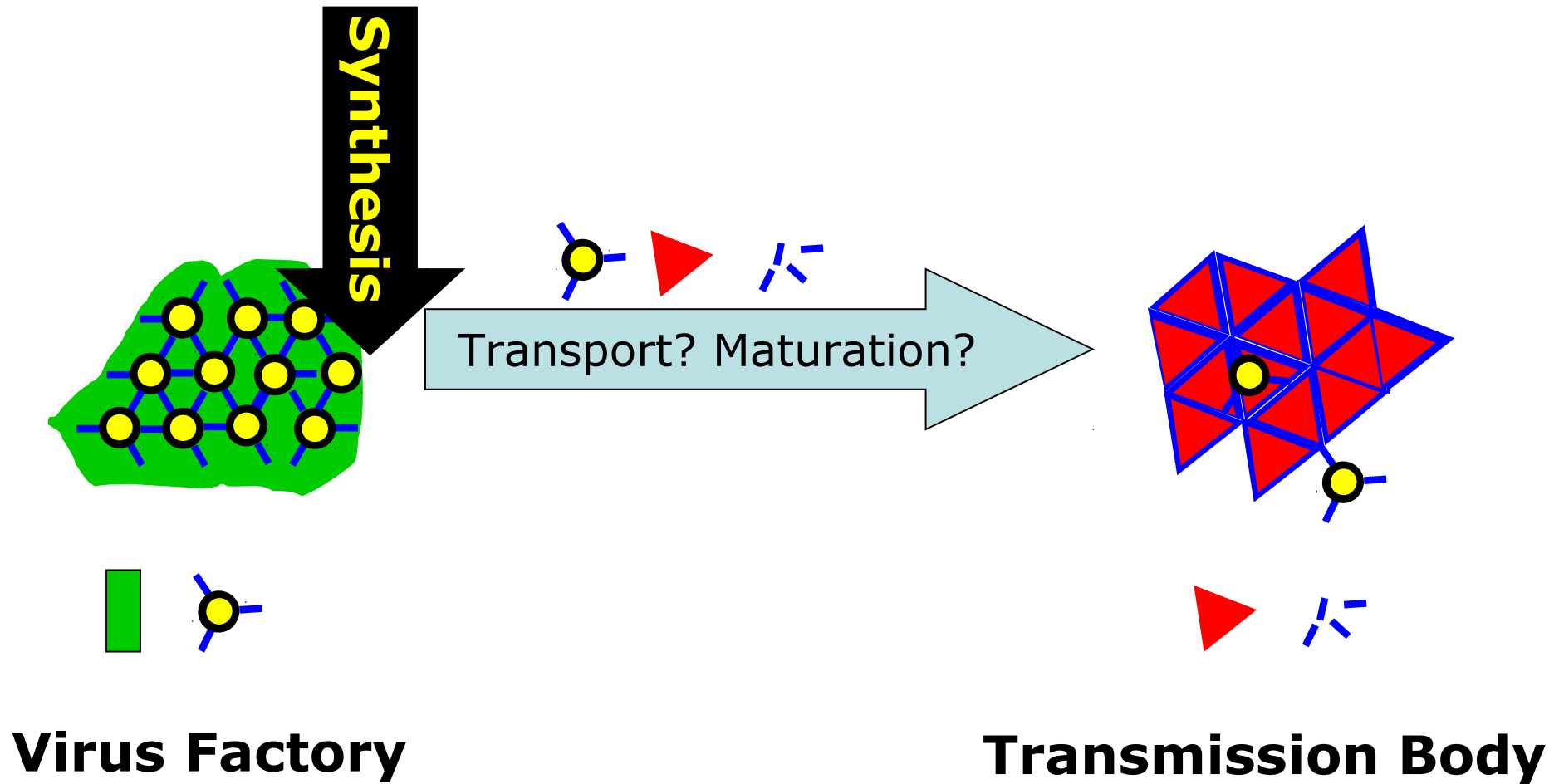


Plant cell

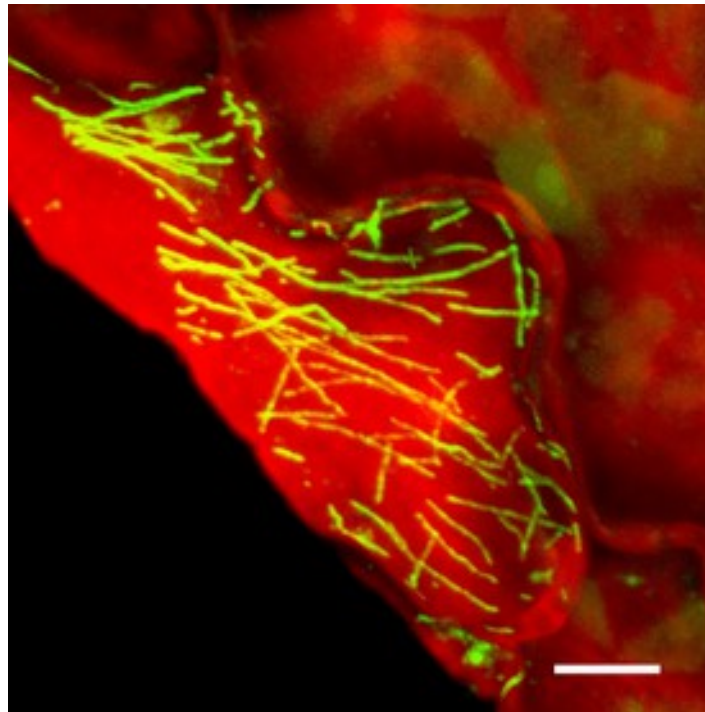
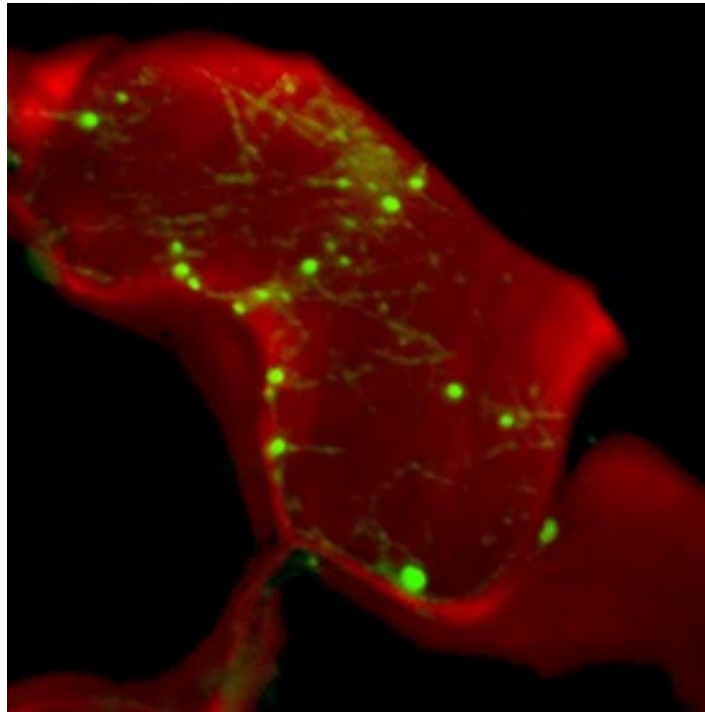
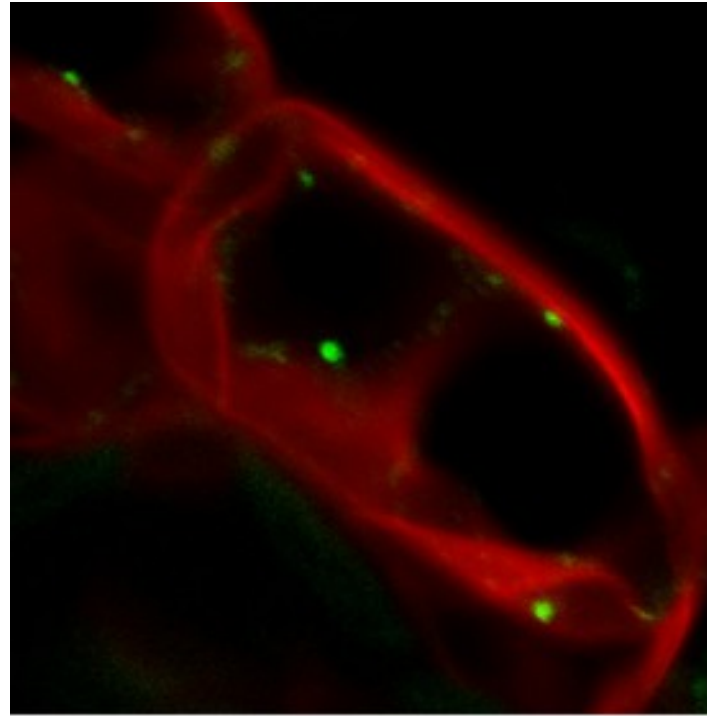
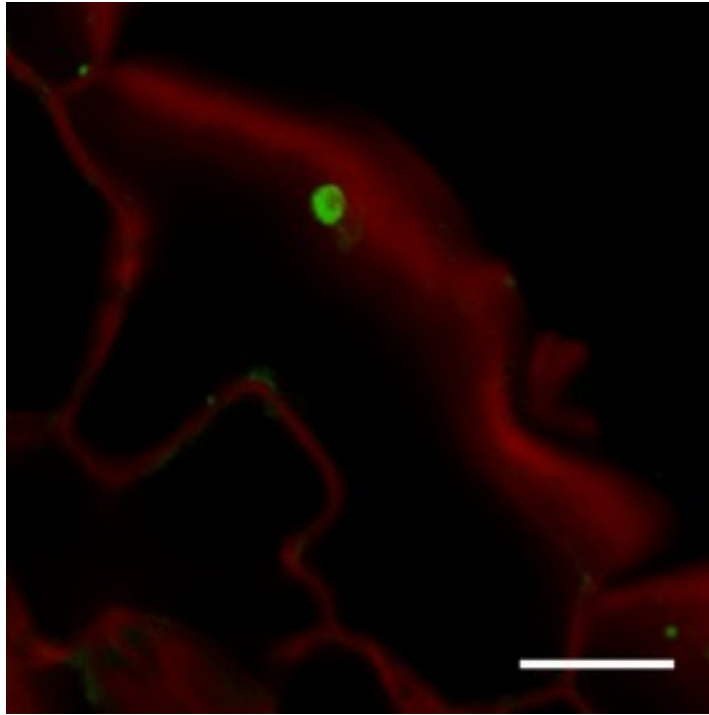
P2, P3 and virions are separated in infected cells



How do Transmission Bodies form?

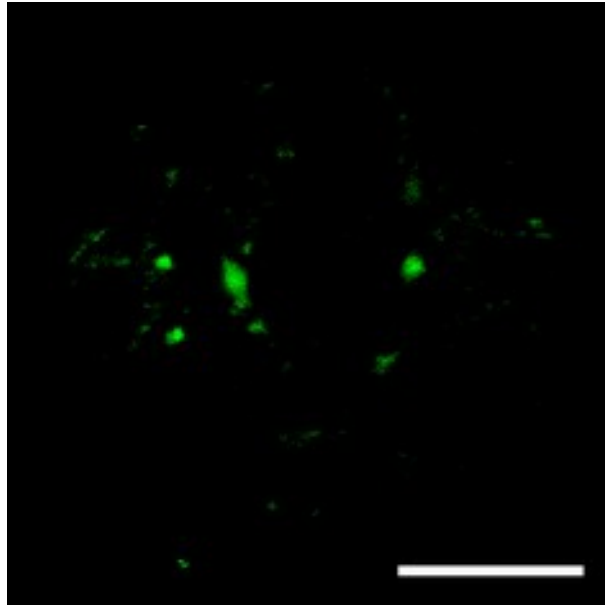


Localisation of P2 in infected leaves

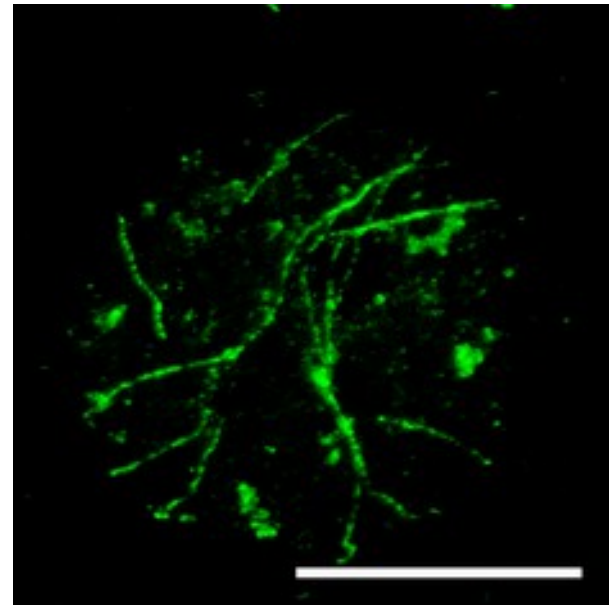


Transfection of protoplasts: Kinetics of Transmission Body formation

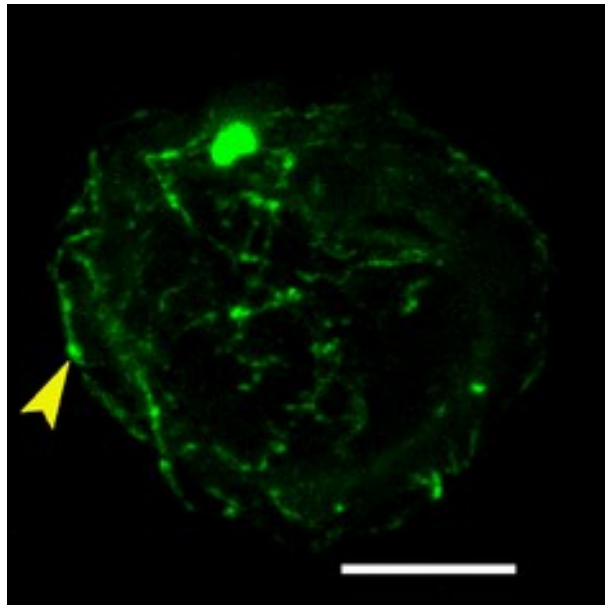
24h



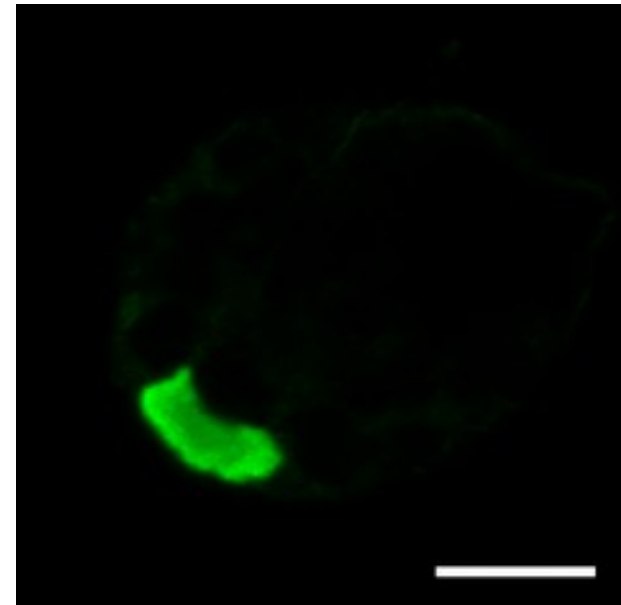
48h



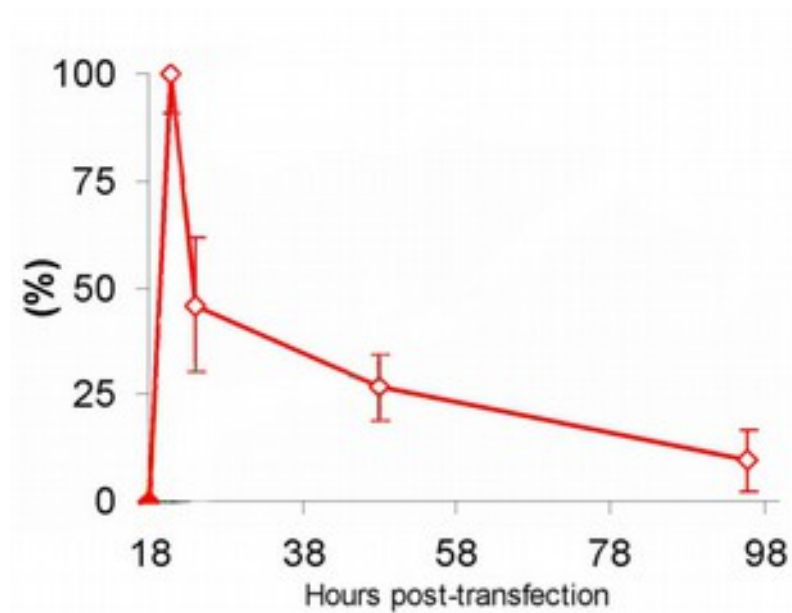
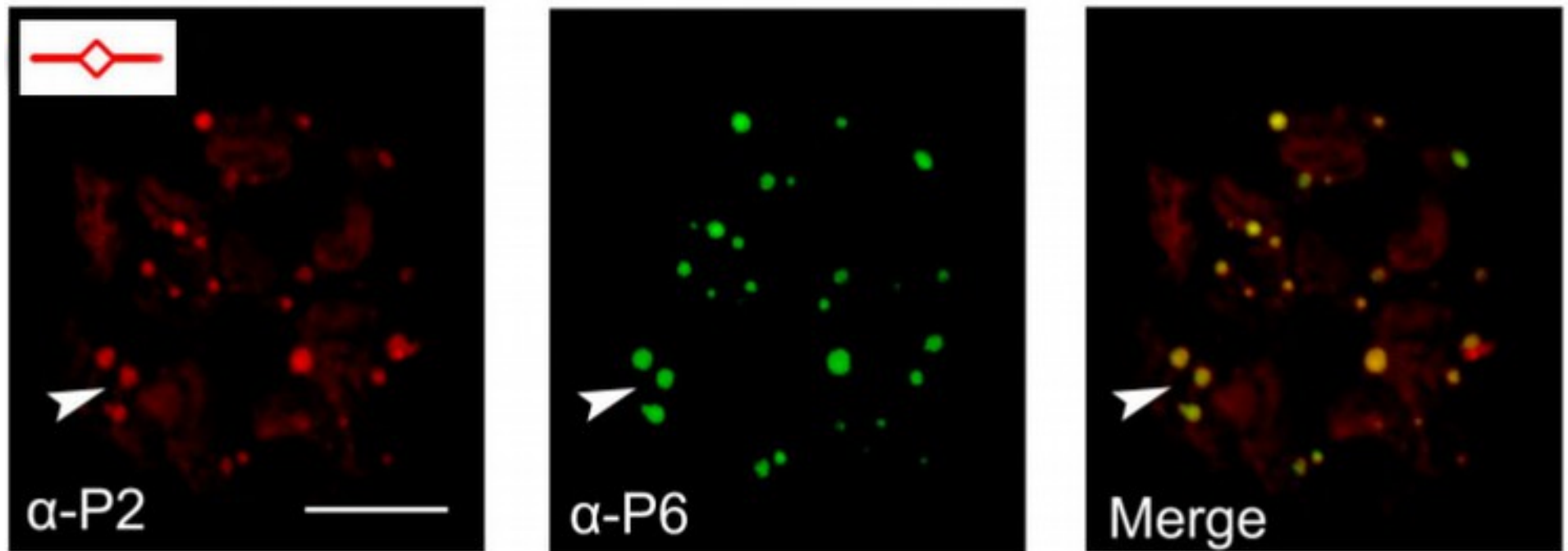
72h



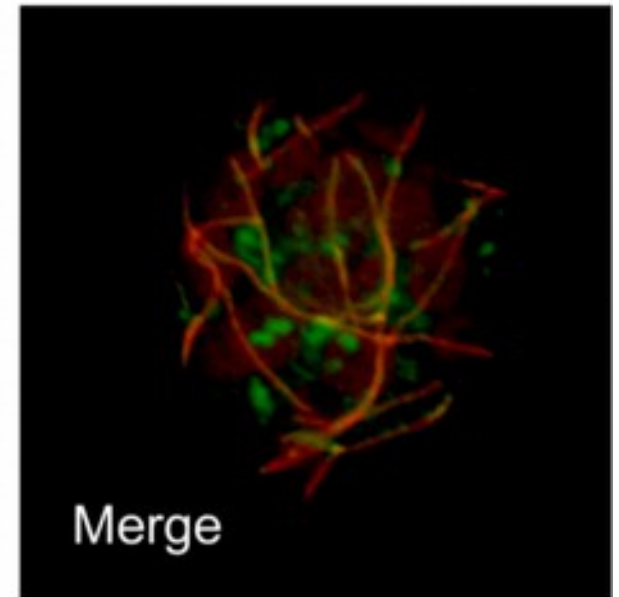
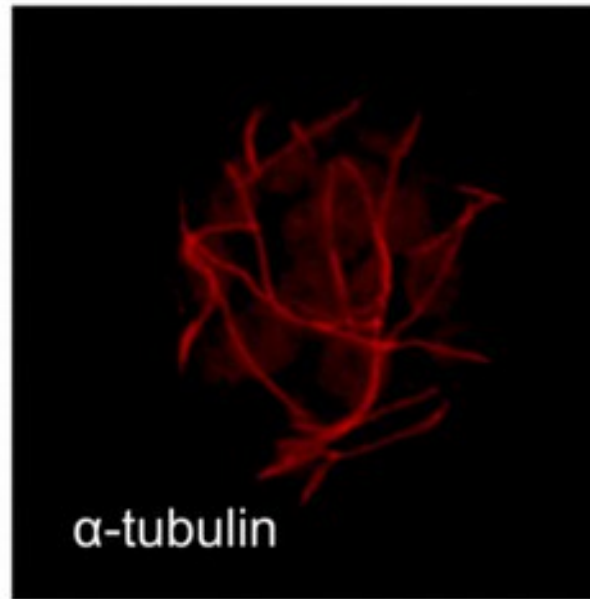
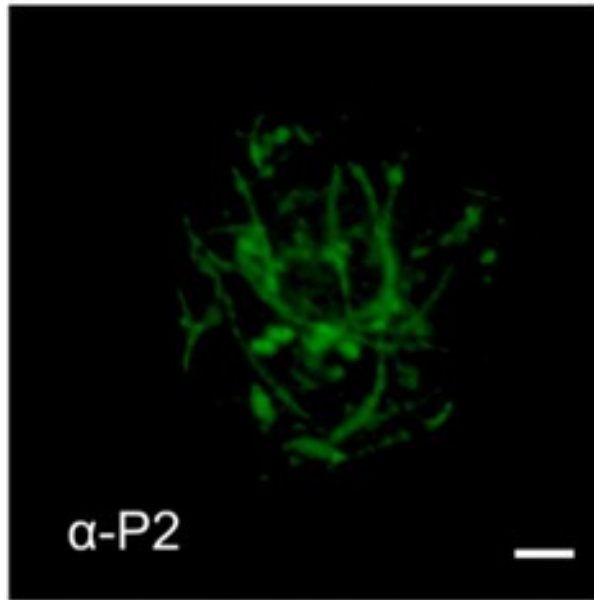
96h



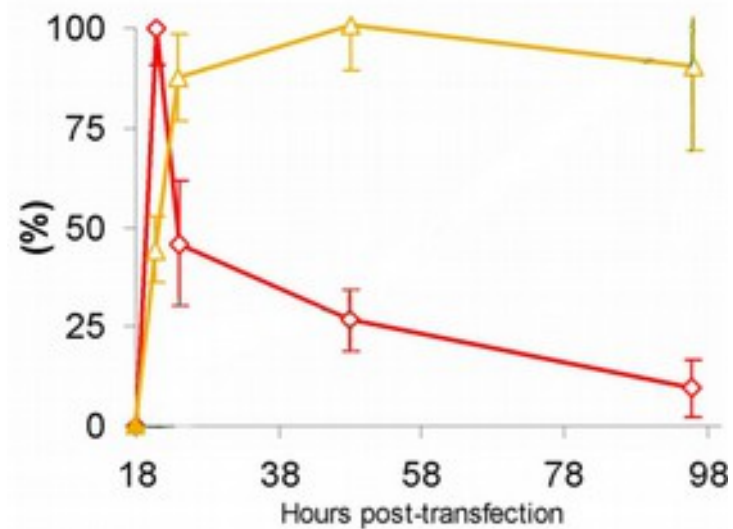
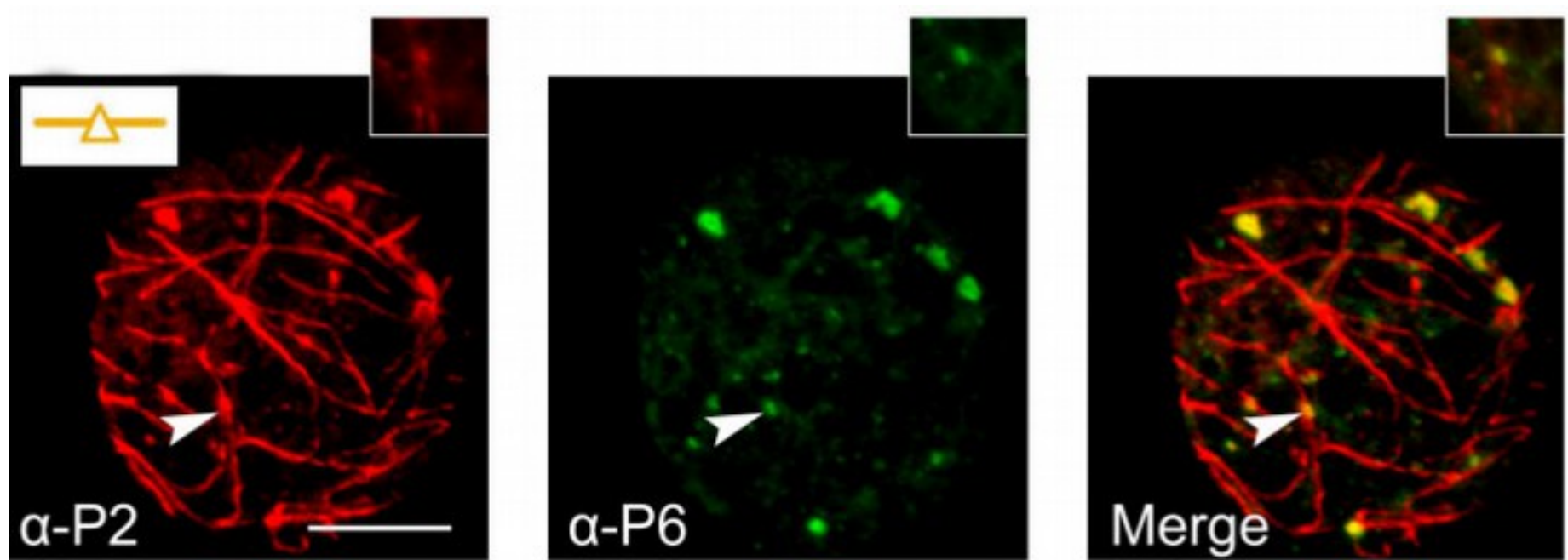
P2 first appears in Virus Factories



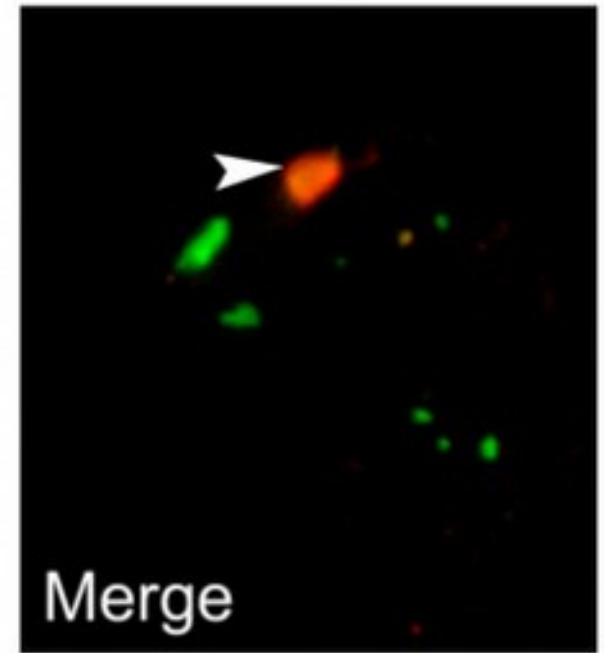
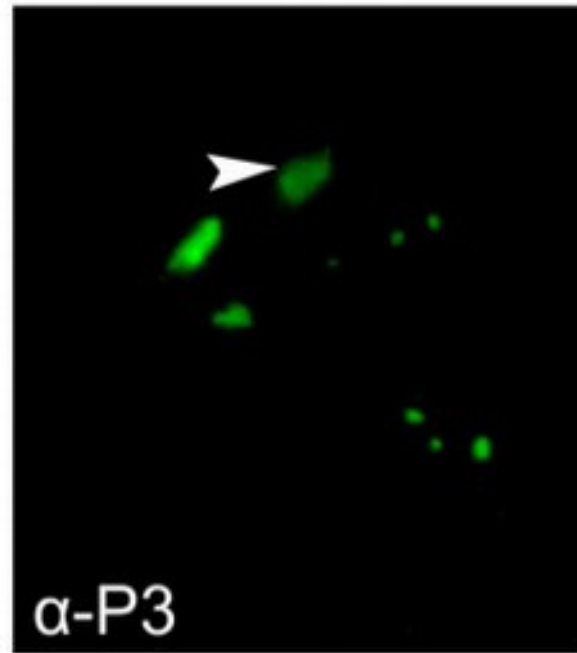
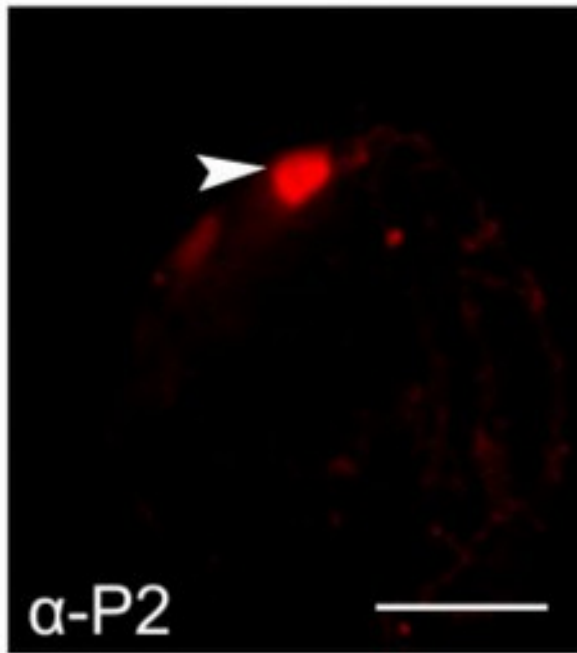
P2 then colocalises with microtubules



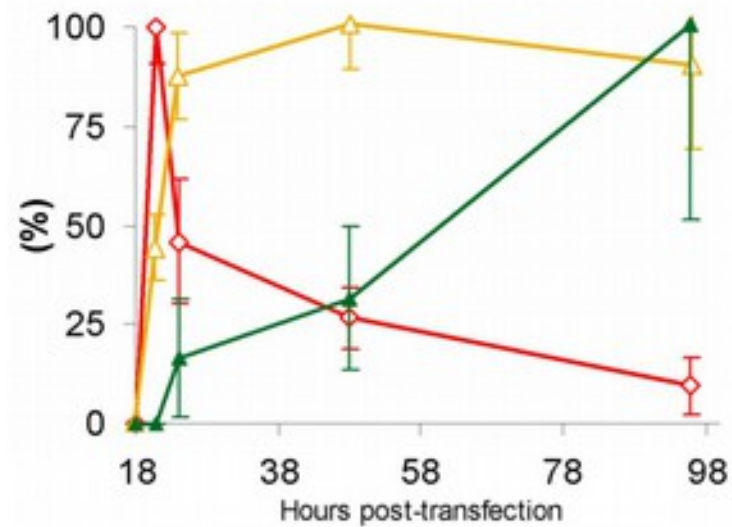
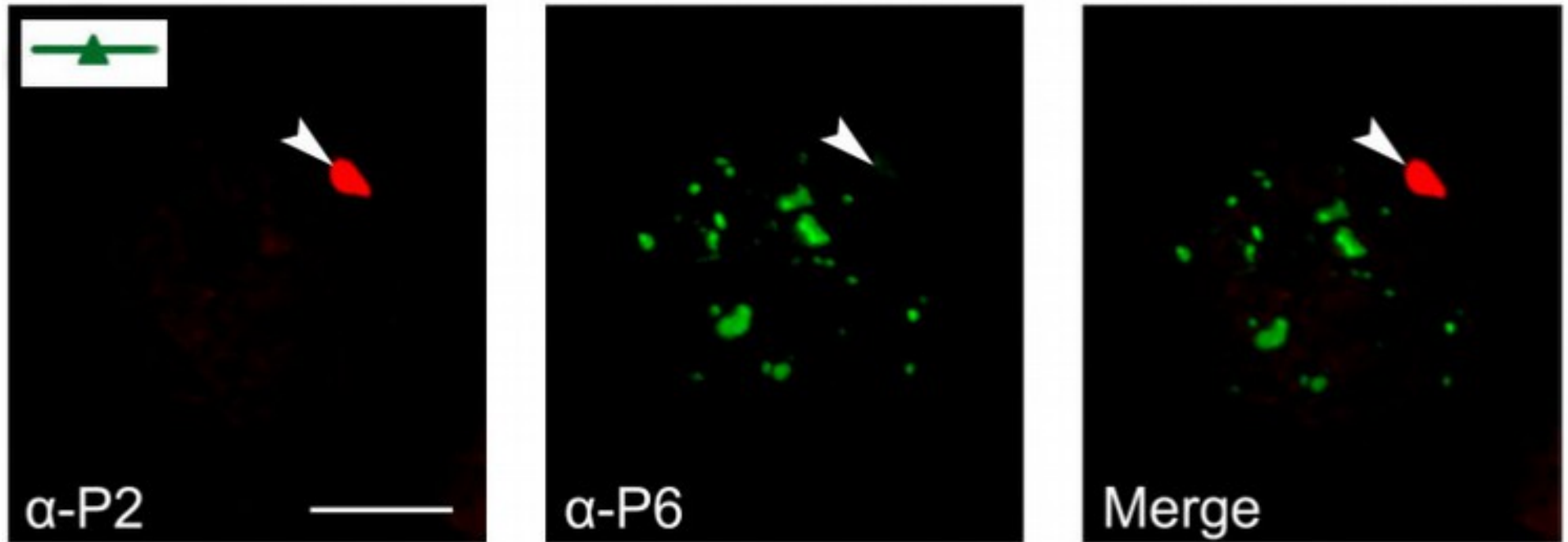
P2 then colocalises with microtubules



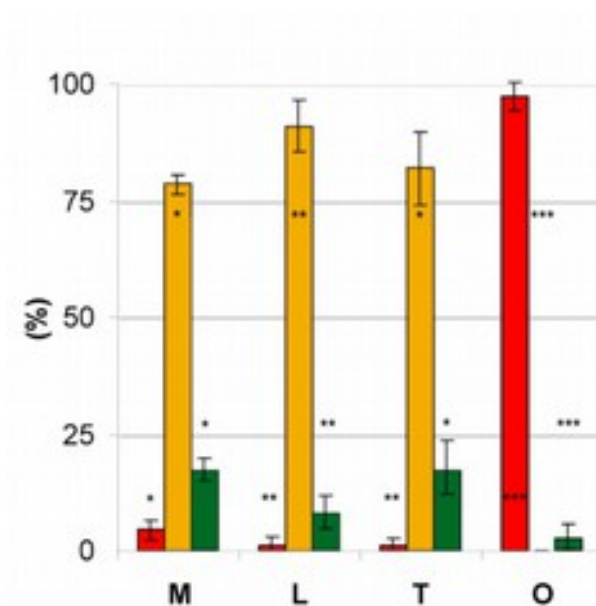
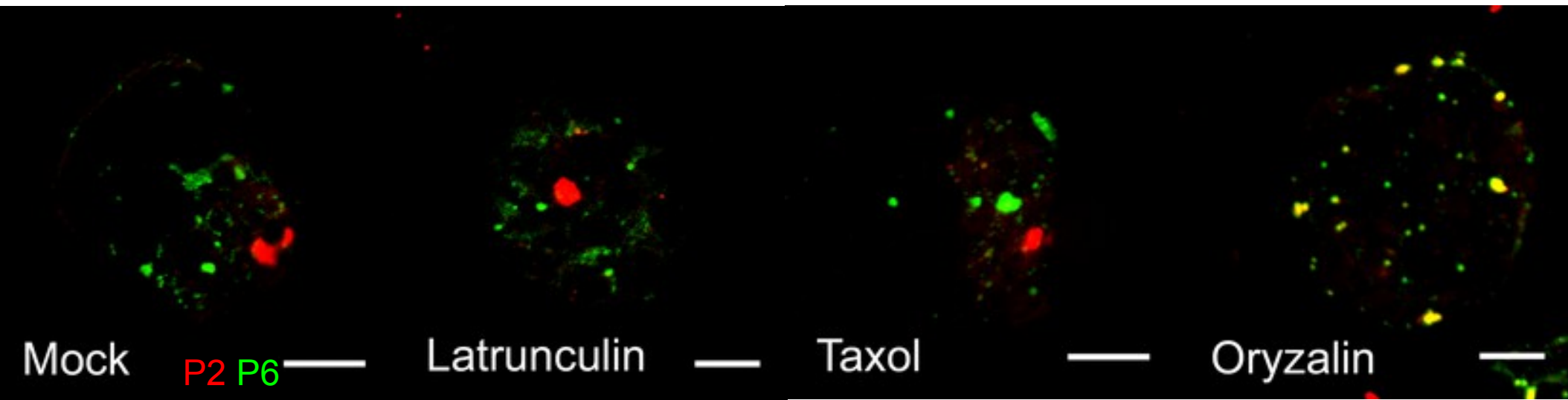
P2 forms a Transmission Body late in infection



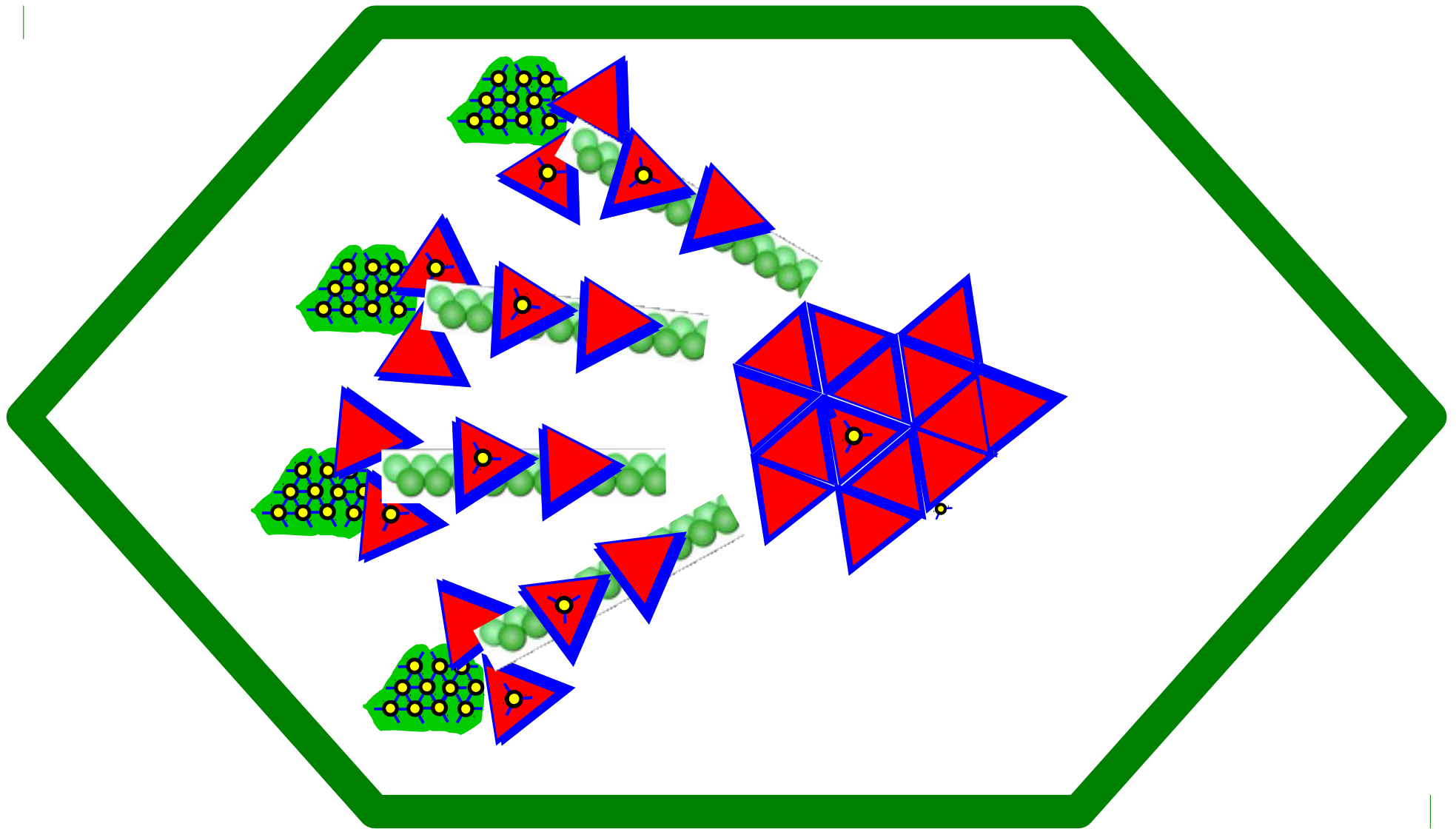
P2 forms a Transmission Body late in infection



Transmission Body formation is microtubule dependent



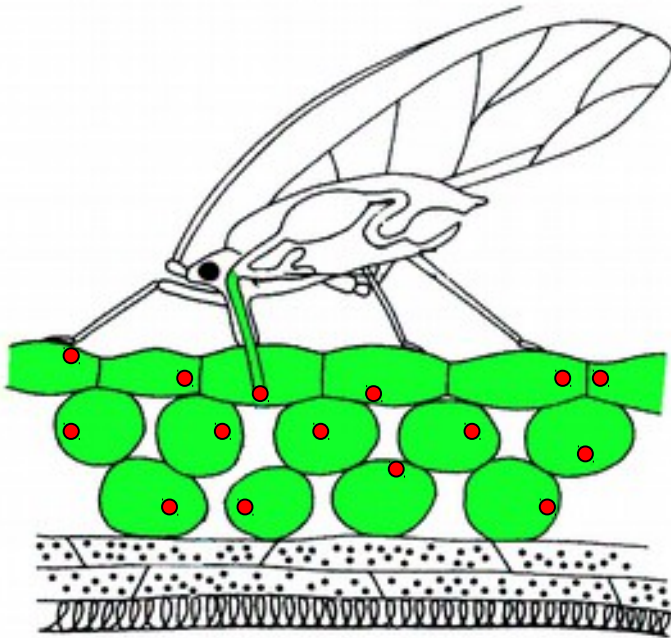
P2 in Virus Factories
P2 on microtubules
P2 in Transmission Bodies



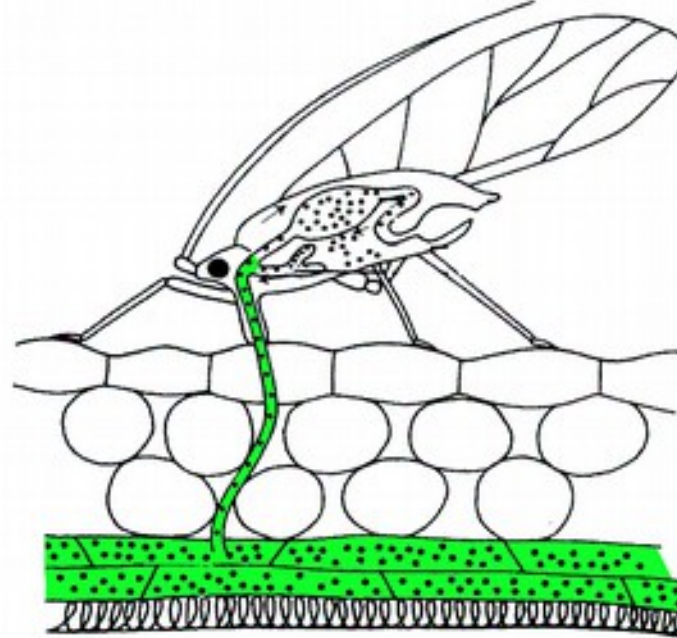
**How are P2, P3, and virions transported?
Why is there only one Transmission Body?**

How does the Transmission Body function?

The process of CaMV acquisition

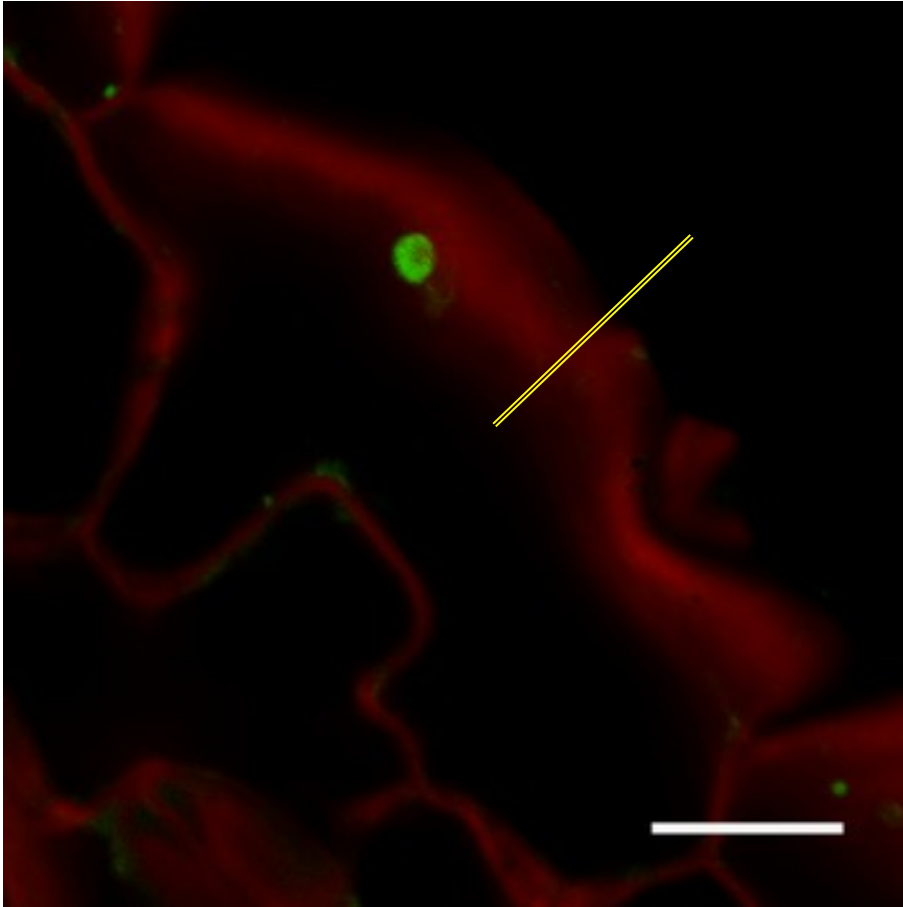


1. Probing



2. Feeding

The process of CaMV acquisition



Transmission Body: ~ 50 fL

Cell: $\sim 5\,000$ fL

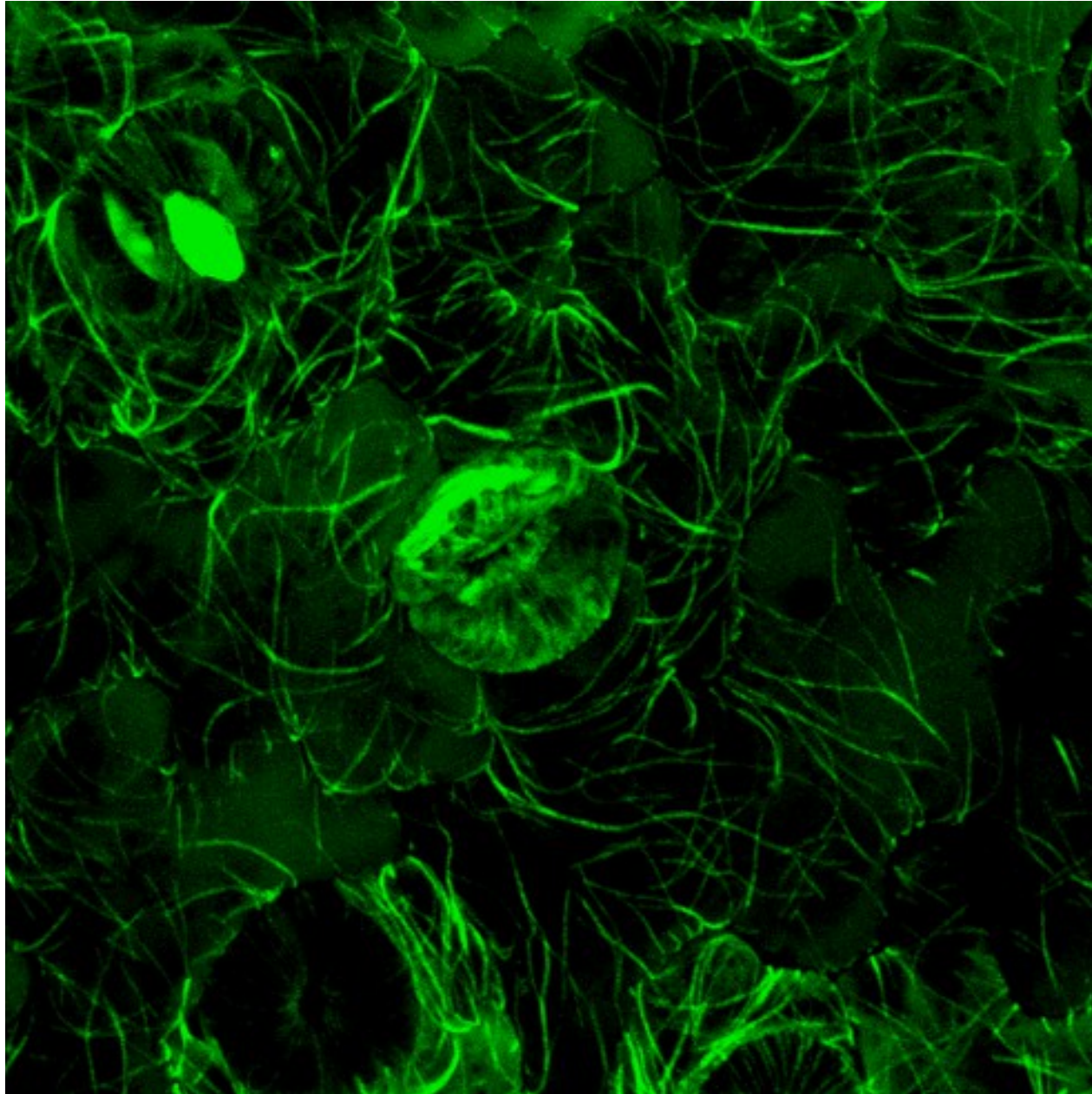
Food canal: ~ 250 fL

Theoretic acquisition rate: 5%

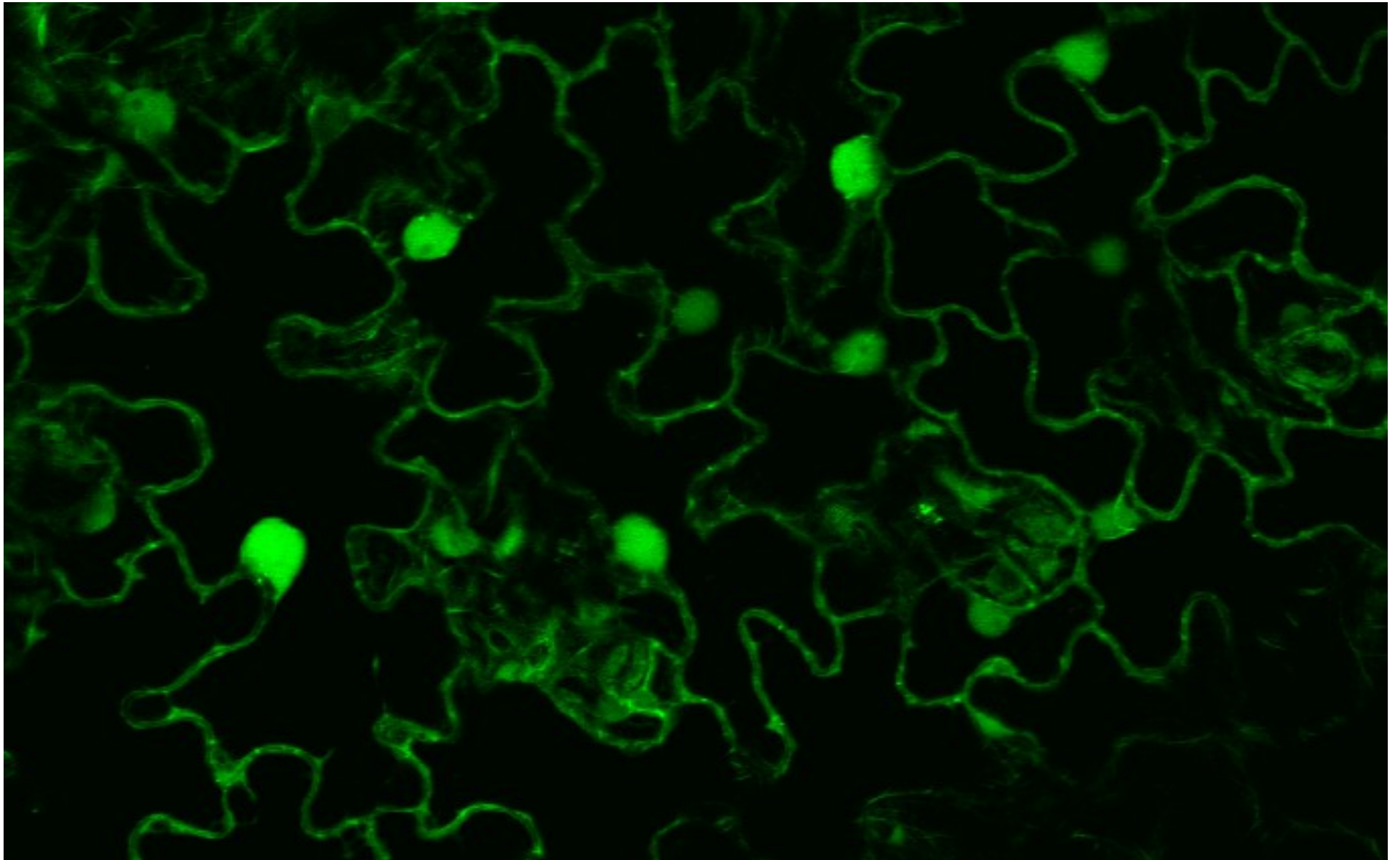
Observed acquisition rate: 30%

How can an aphid acquire the CaMV during a single probing puncture?

Infection of transgenic *Arabidopsis*

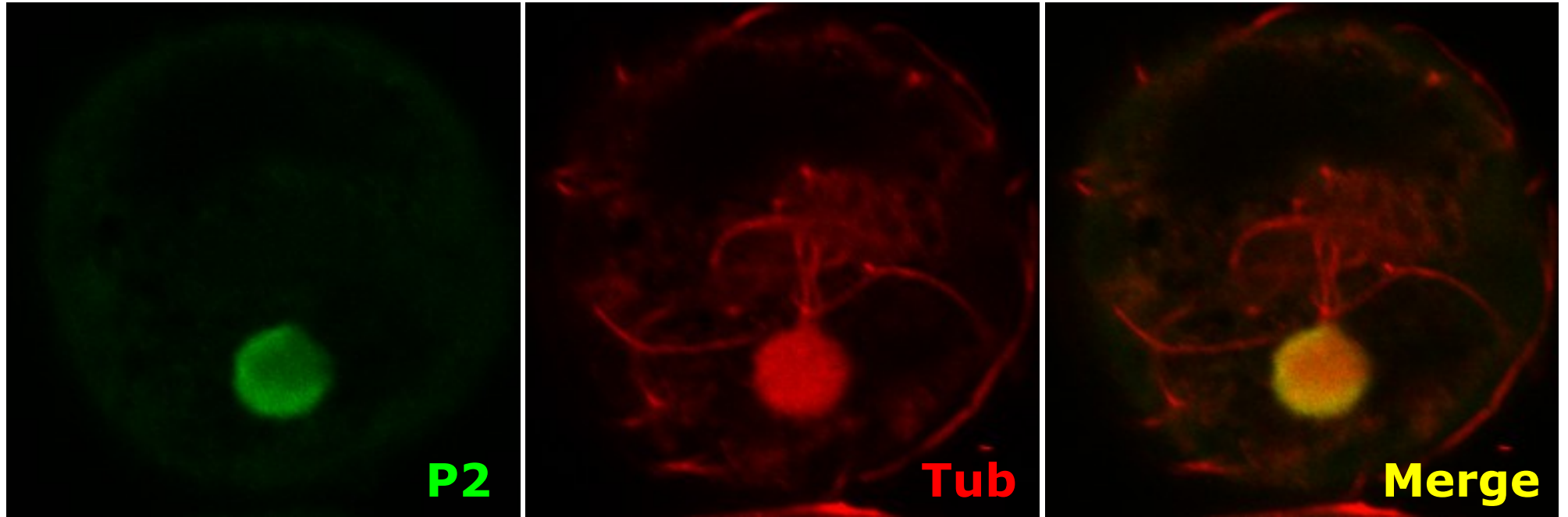


A. thaliana* GFP::*TUA6
not infected



***A. thaliana* GFP::*TUA6*
infected with CaMV**

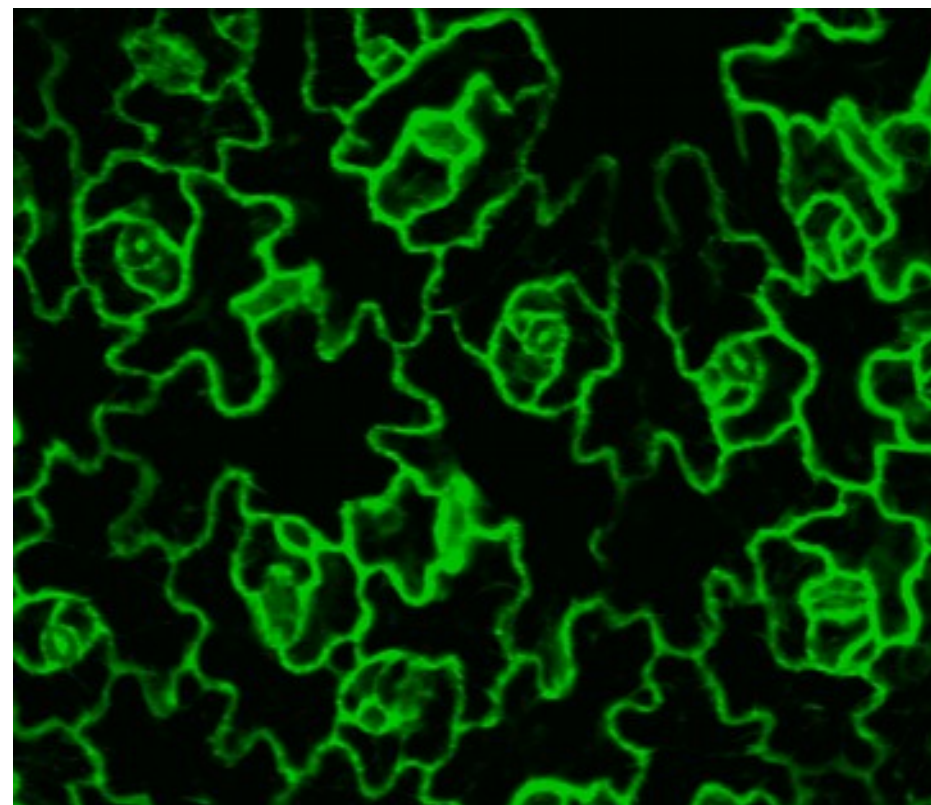
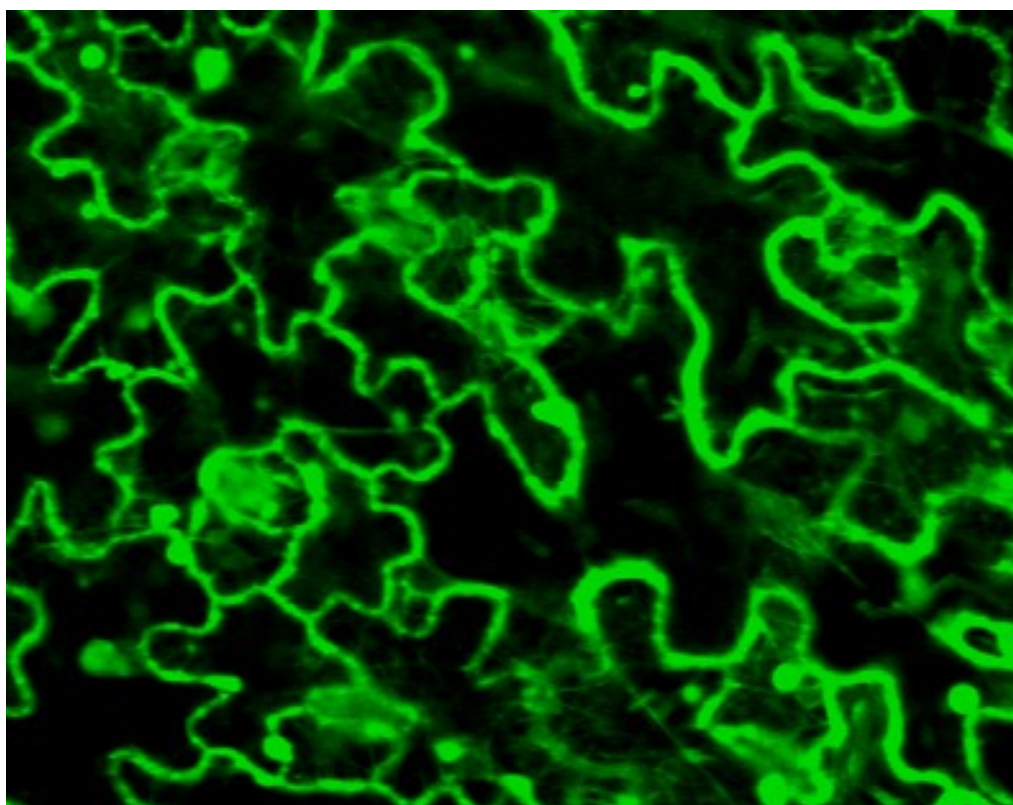
Transmission Bodies in living tissues may contain tubulin



Immunofluorescence:

Tubulin and P2 colocalise in Transmission Bodies

However, not always...



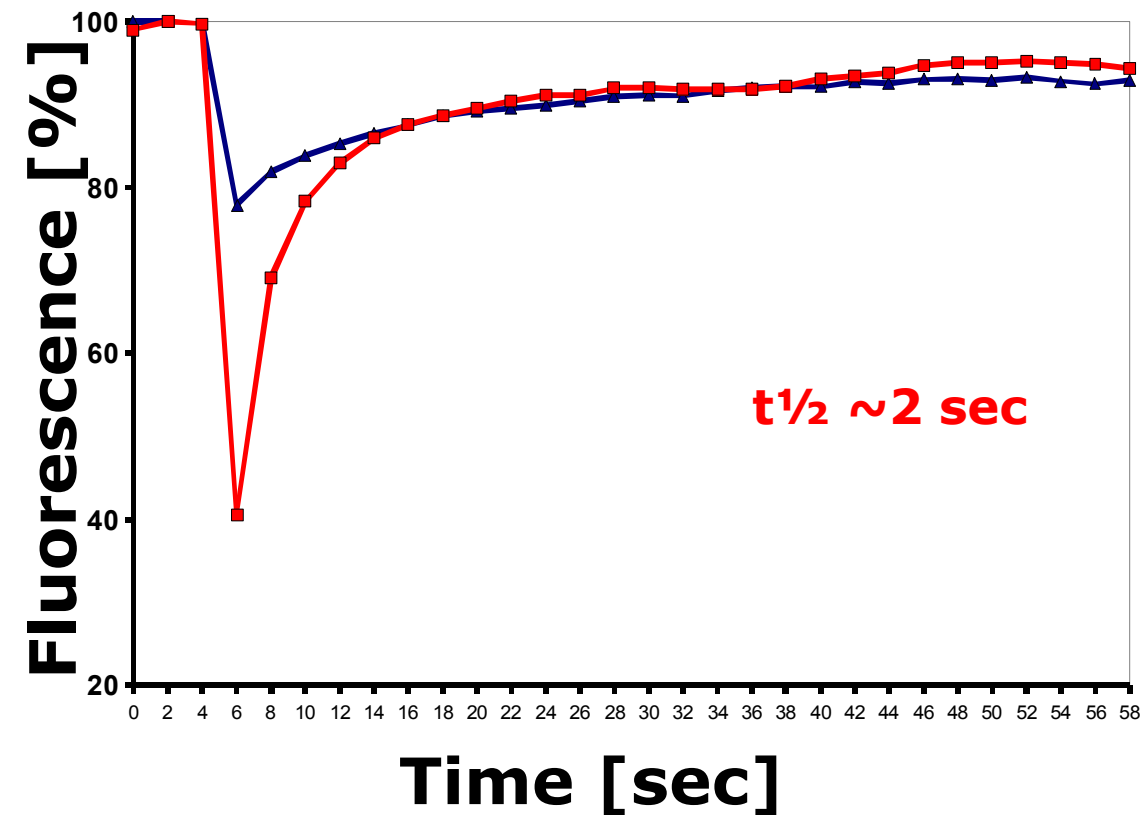
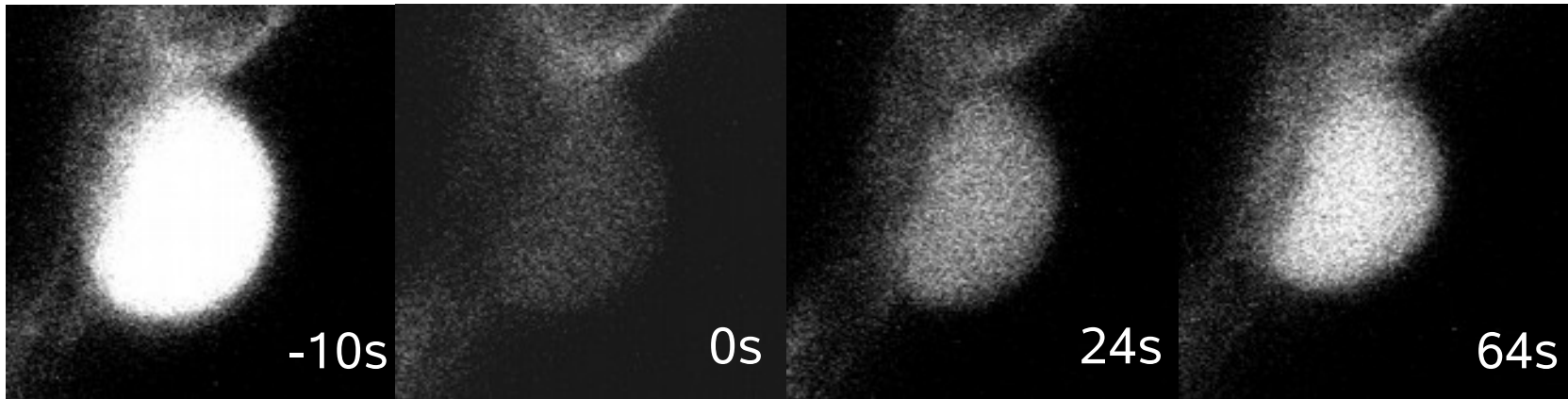
***A. thaliana* GFP::*TUA6*
infected with CaMV**

Tubulin is only sometimes present in Transmission Bodies

- Is tubulin a dynamic component of Transmission Bodies?**
- What causes tubulin influx in Transmission Bodies?**
- Does tubulin influx influence transmission efficiency?**

Dynamics of tubulin in Transmission Bodies

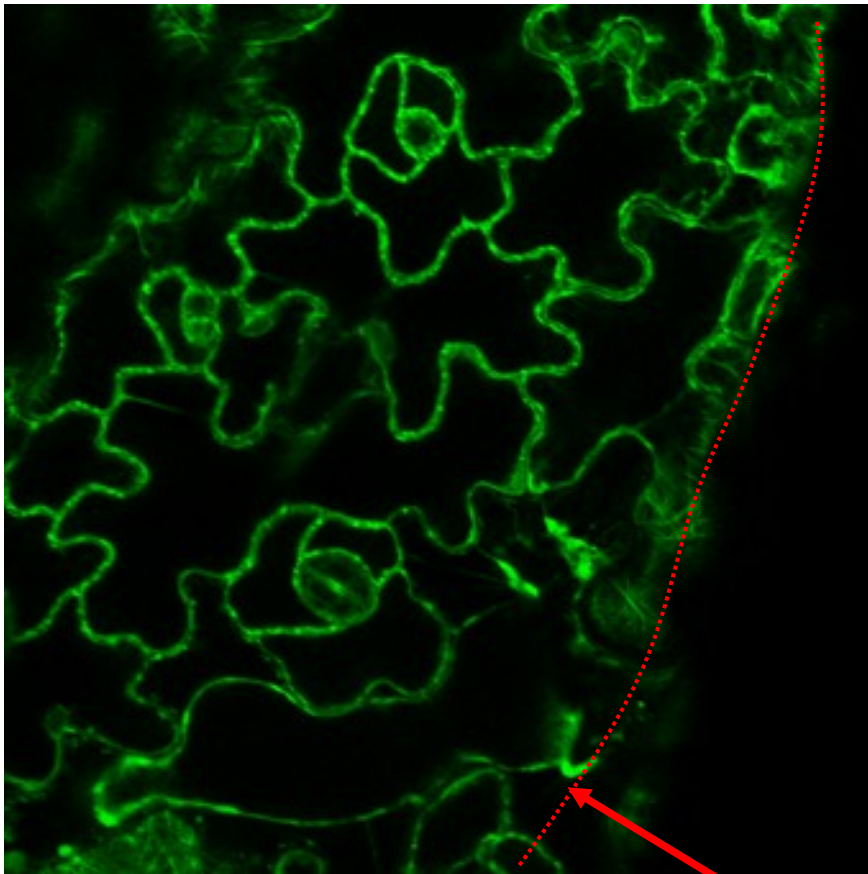
FRAP on GFP::TUA in Transmission Bodies



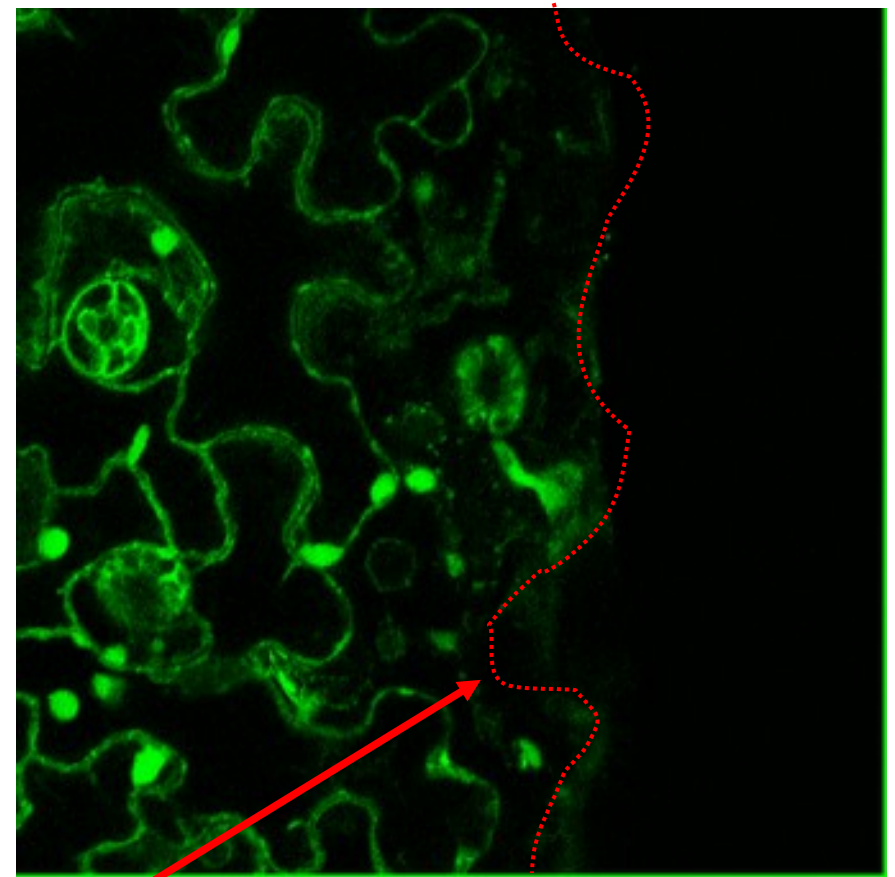
→ soluble tubulin

What causes tubulin entry in the Transmission Body?

Observation:



healthy

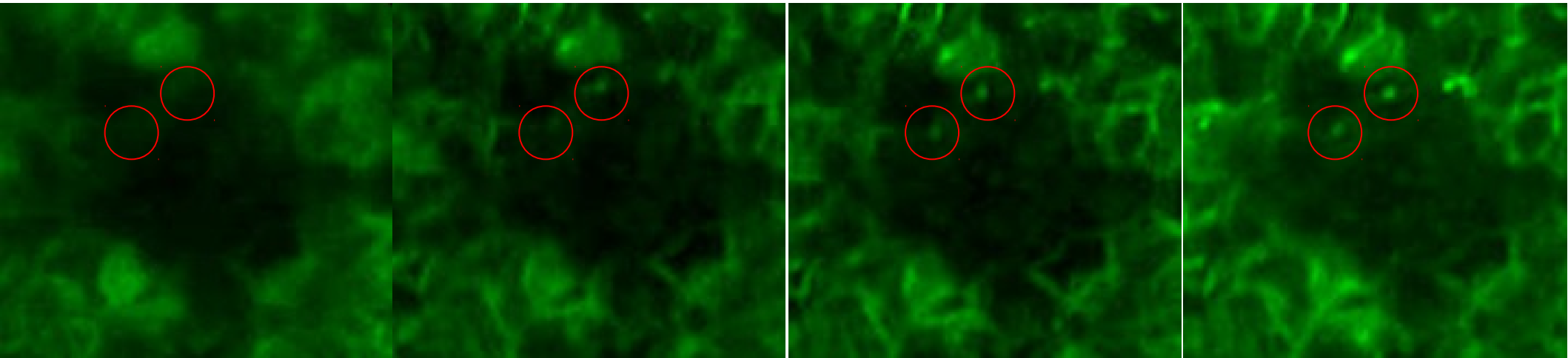


CaMV

Cutting edge

Does wounding stress cause entry of tubulin in the Transmission Body?

Artificial wounding of leaves



0 sec

2 sec

4 sec

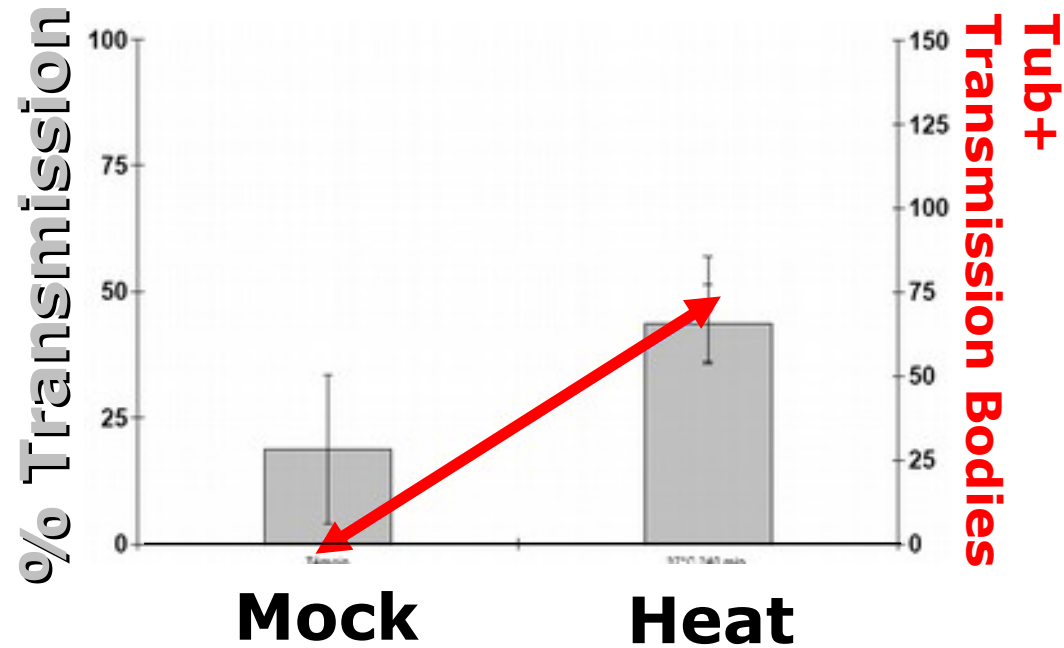
8 sec

**Wounding causes fast entry of tubulin in the Transmission Body
→ Compatible with duration of aphid probing punctures (seconds)**

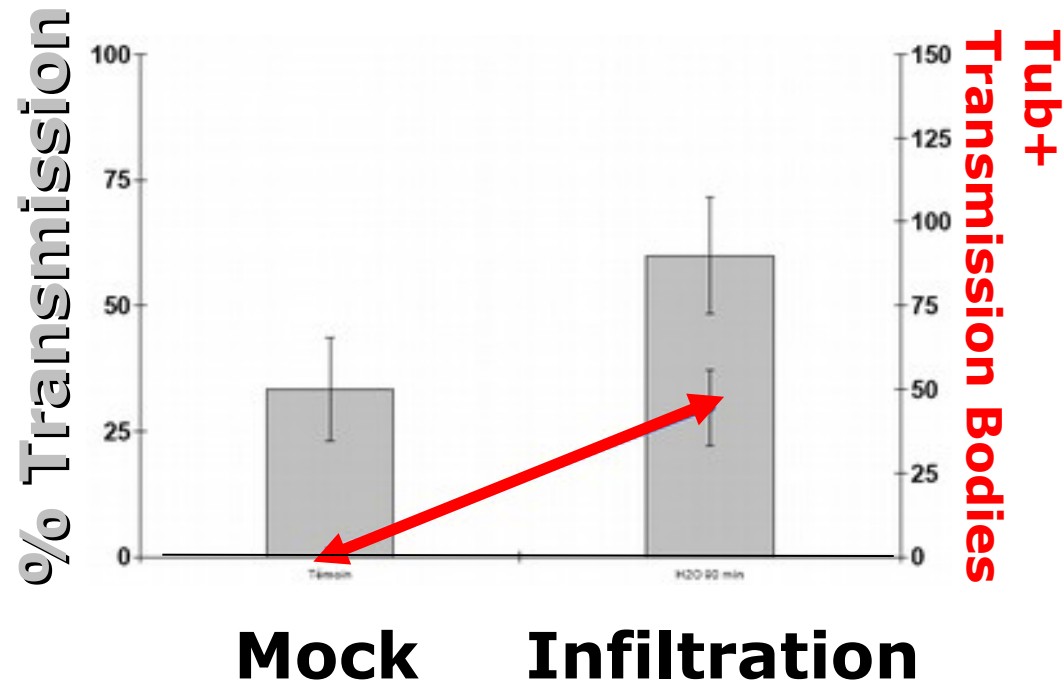
Does tubulin influx influence transmission efficiency?

Tubulin influx “activates” the Transmission Body

Heat stress



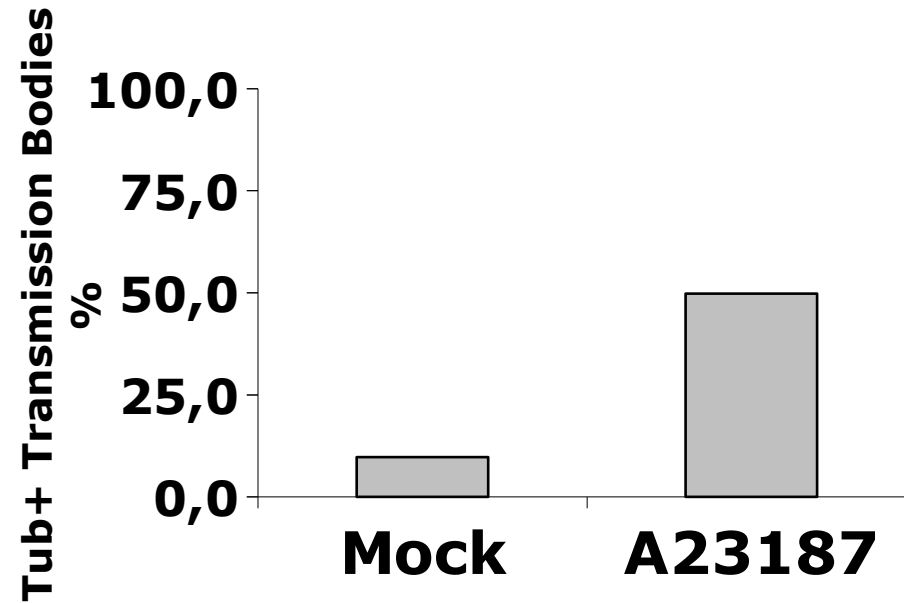
Infiltration stress



Tubulin influx “activates” the Transmission Body

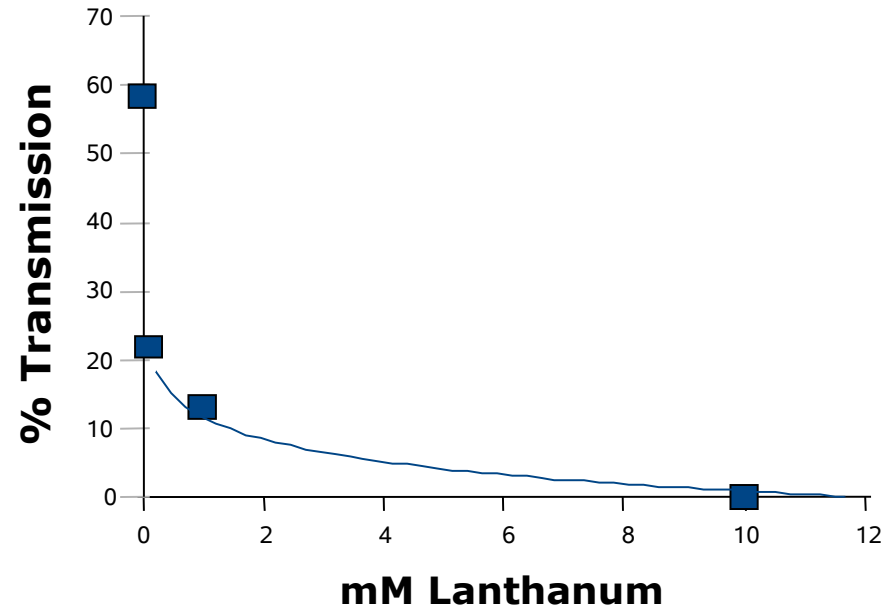
How is the stress perceived (Signal transduction)?

Ca Ionophor

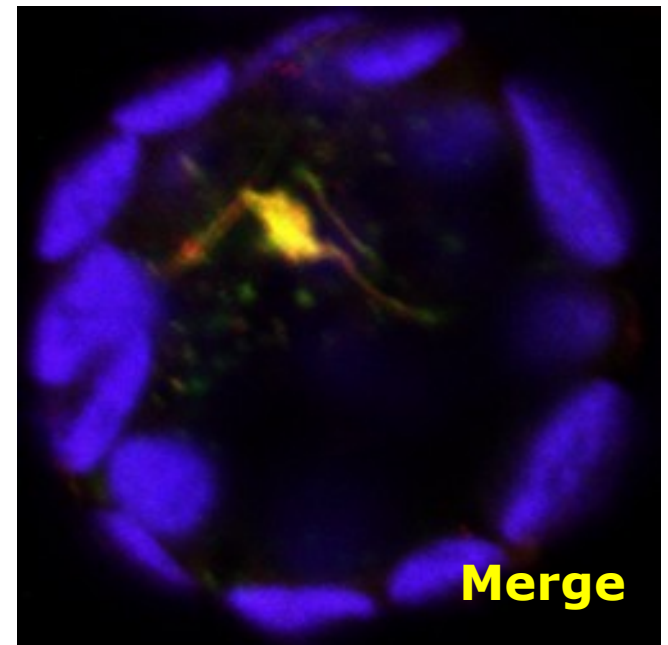
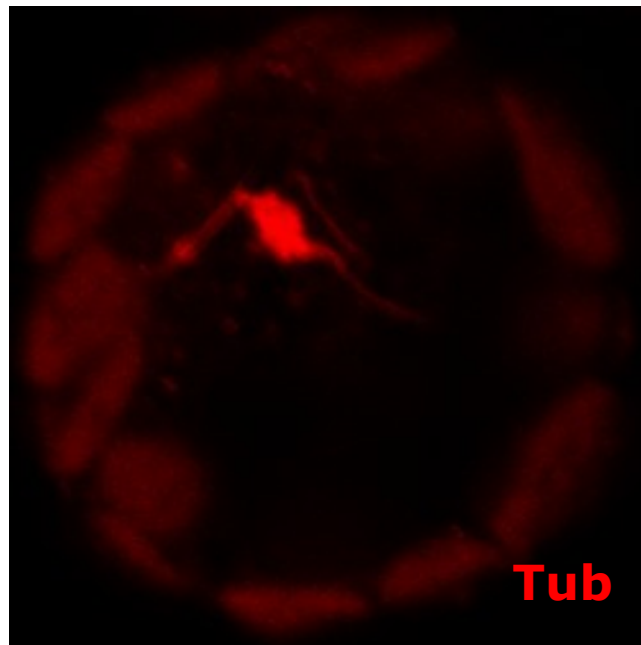
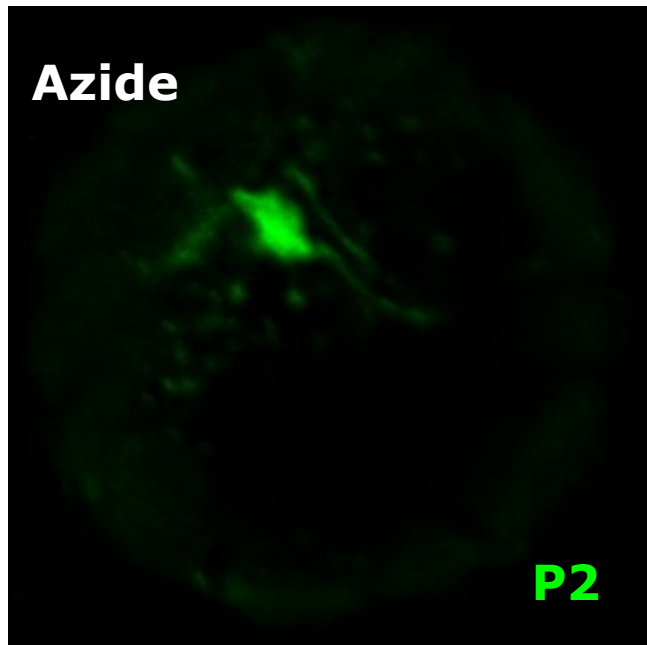
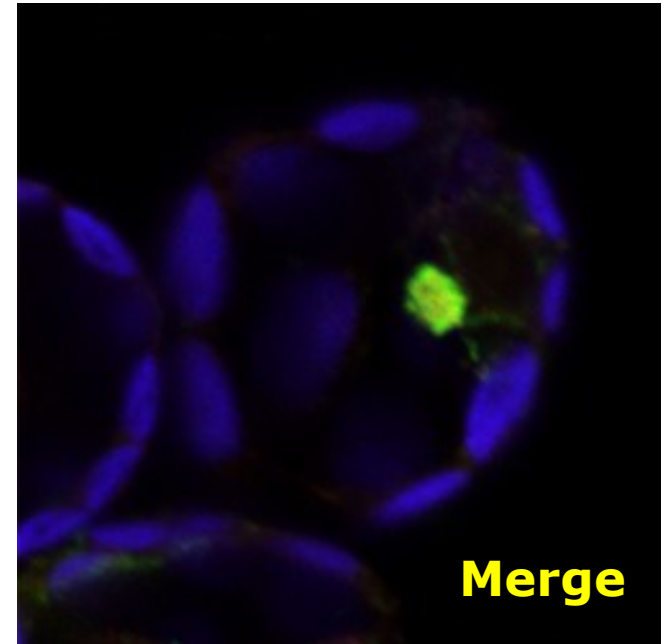
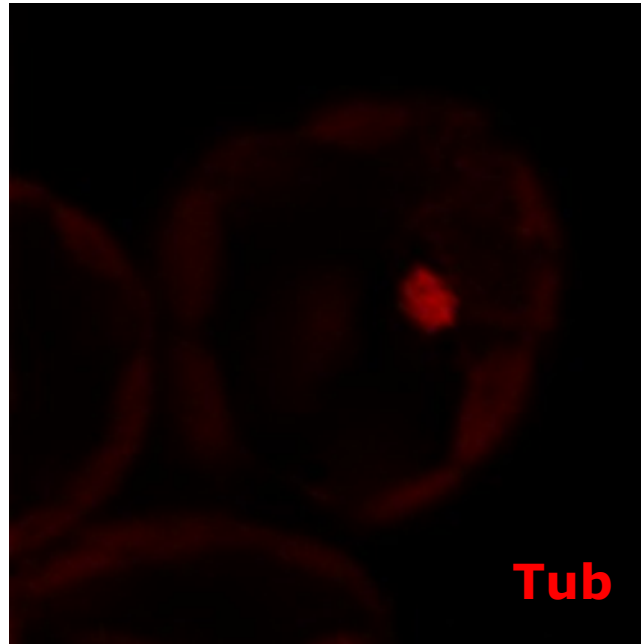


→ **Calcium**

Ca Channel blocker

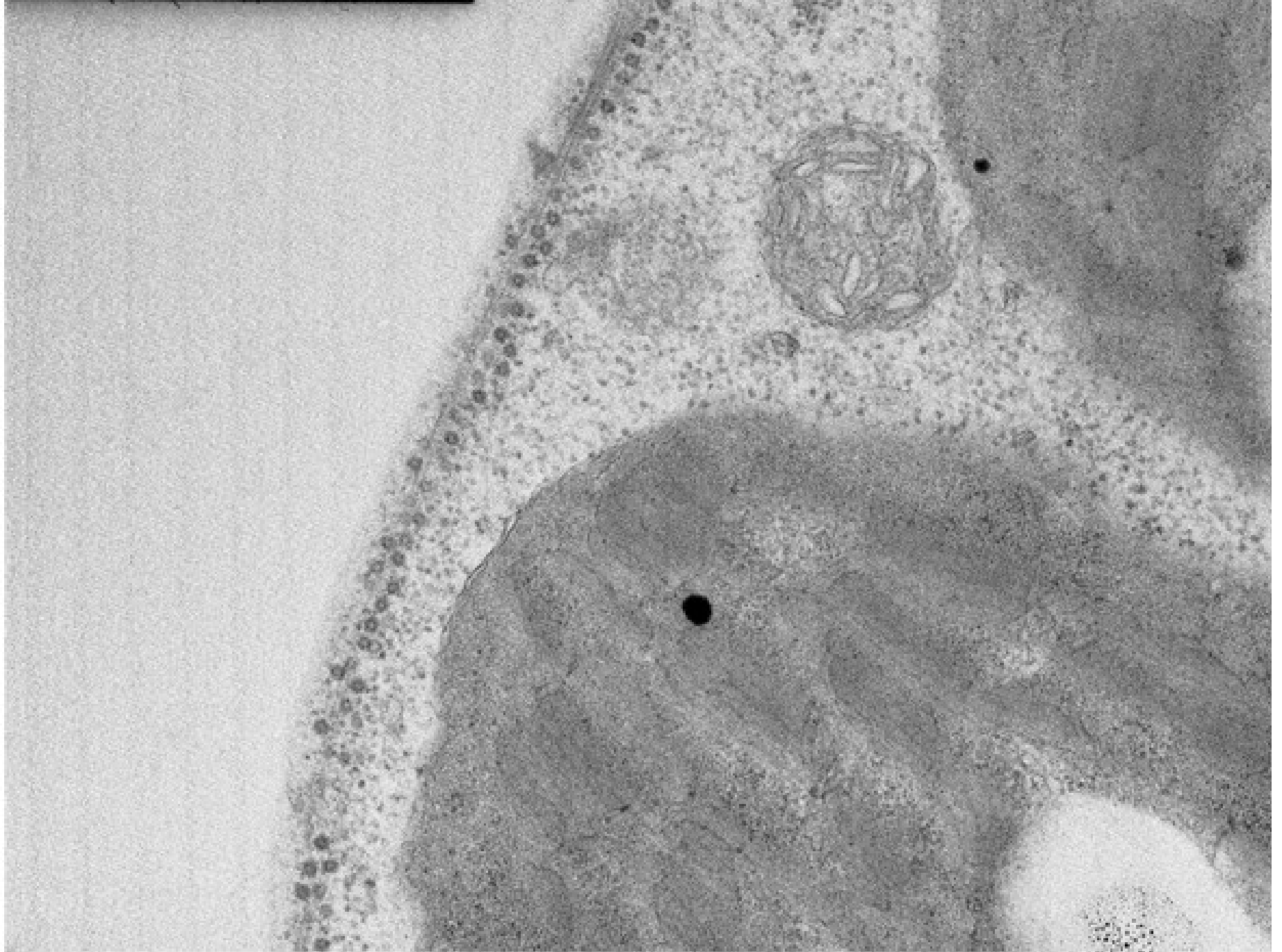


Is there a second step in Transmission Body activation?

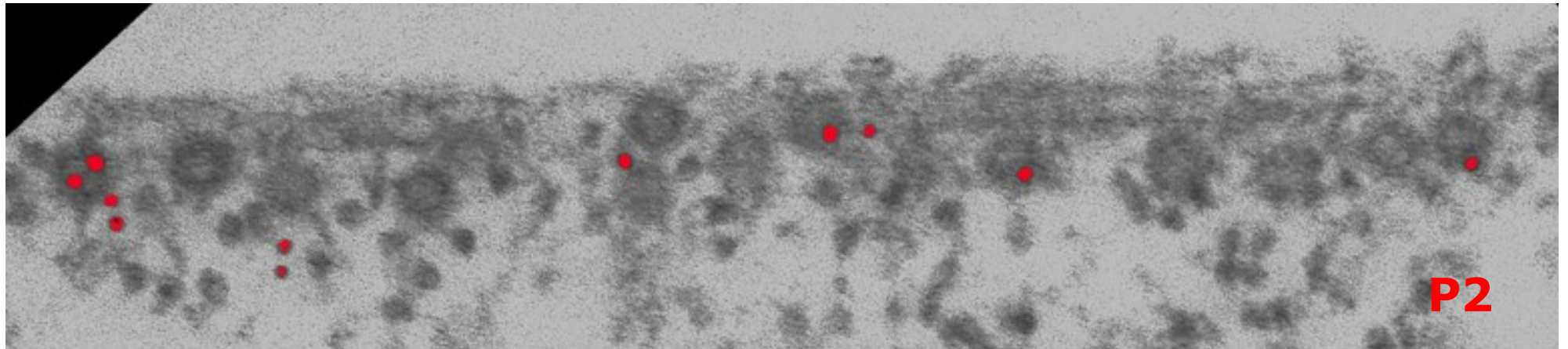
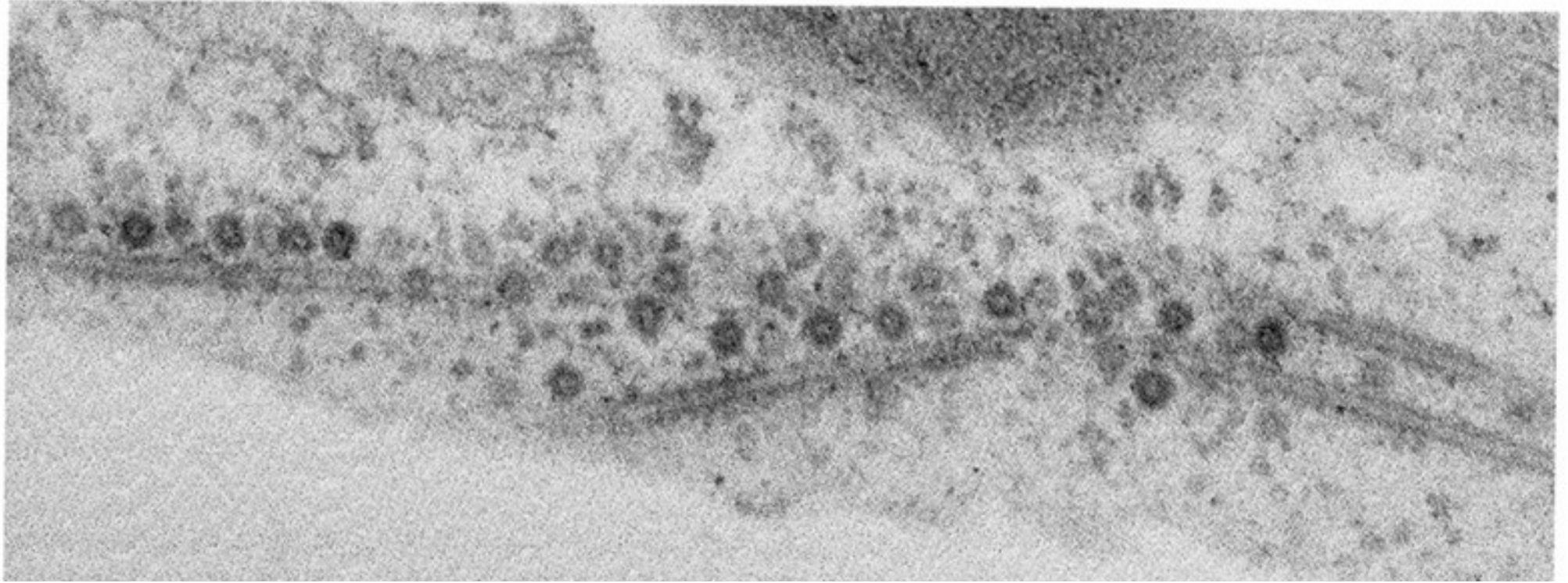


Immunofluorescence

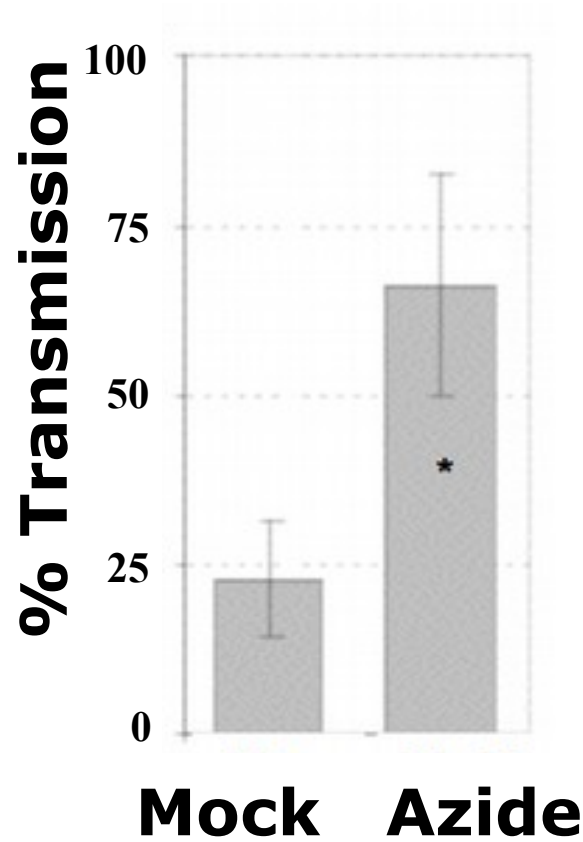
Is there a second step in Transmission Body activation?



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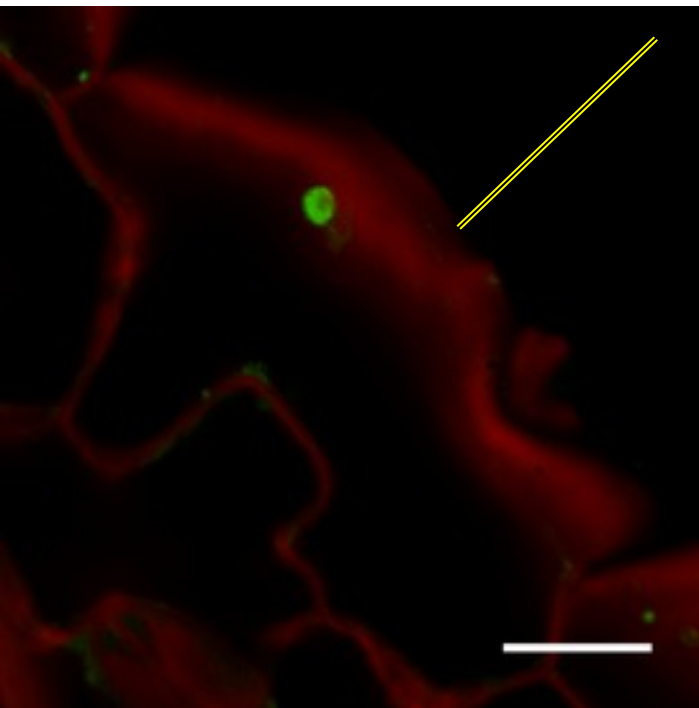


Is there a second step in Transmission Body activation?

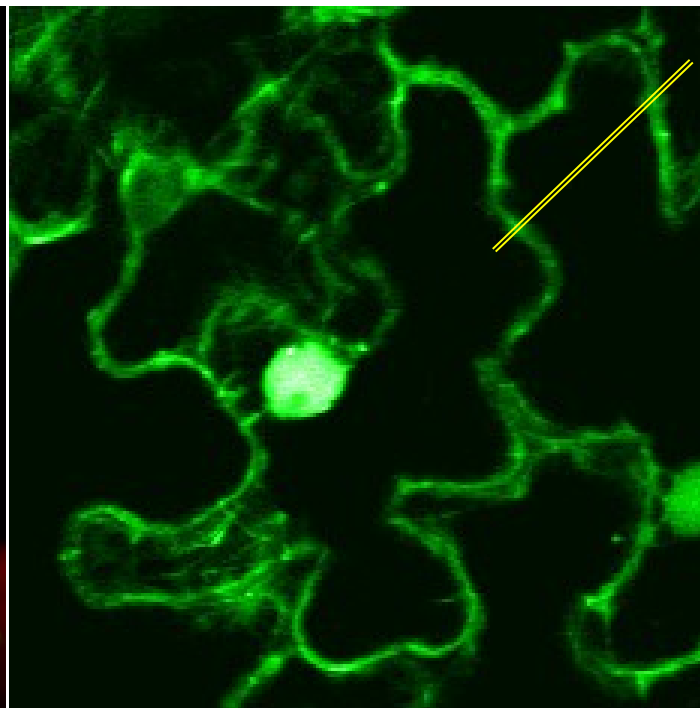


Model: CaMV “senses” the vector and prepares for transmission

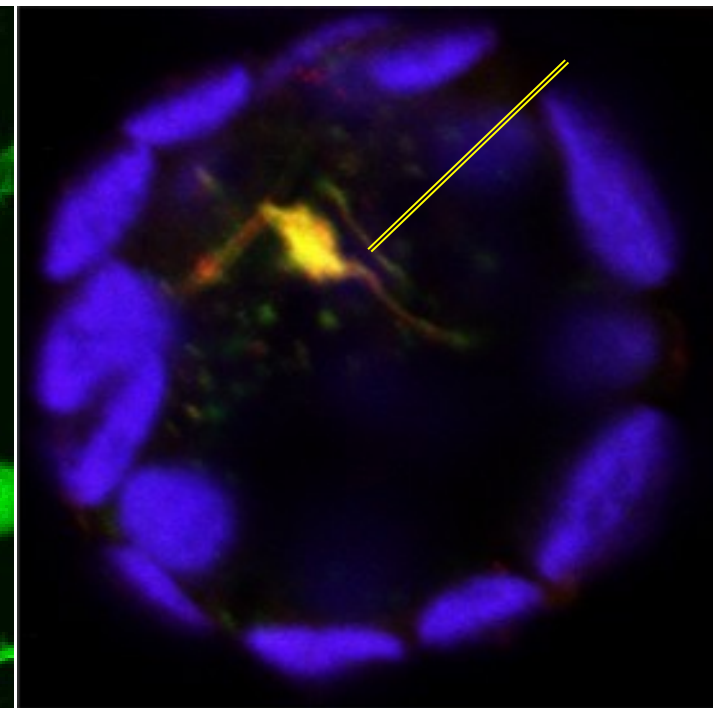
- Puncture → Wounding or chemical stress**
- Calcium signaling cascade**
 - Tubulin influx**
 - Dispersion of P2 and virus throughout the cell**



“stand-by”



“activated”



“competent”



Merci!